Placenta 103 (2021) 104–119

Contents lists available at ScienceDirect

Placenta

journal homepage: http://www.elsevier.com/locate/placenta

Update review on five top clinical applications of human amniotic membrane in regenerative medicine

Aida Rezaei Nejad [a], Amir Ali Hamidieh [b,a], Mohammad Amir Amirkhani [a,**], Mahsa Mollapour Sisakht [a,c,*]

[a] Stem Cell and Regenerative Medicine Center of Excellence, Tehran University of Medical Sciences, Tehran, Iran
[b] Pediatric Cell Therapy Research Center, Tehran University of Medical Sciences, Tehran, Iran
[c] Department of Biochemistry, Erasmus University Medical Center, Rotterdam, the Netherlands

ARTICLE INFO

Keywords:
Amniotic membrane
Regenerative medicine
Clinical trial
Cell therapy

ABSTRACT

Due to the increasing number of studies performed in the field of regenerative medicine during the last two decades, more analytic studies are still needed to clarify the future prospect of this area of science. The main aim of this research was to review the clinical applications of human Amniotic membrane in the field of regenerative medicine critically. Furthermore, in the light of increasing numbers of available products derived from amniotic membrane, we aimed look in depth to see whether regenerative medicine research strategies have a place in the clinical setting. More specifically, in the present study, we attempted to provide insight on developing the new indication for more research and in the next step, for market leaders companies to expand cost-effectiveness of new derived AM products. 20 companies or distributers have offered some commercial products in this field. Survey on more than 90 clinical trials in last five years showed dermatology (and more specific wound healing), orthopedic, and ophthalmology are heavily biased toward multibillion dollar industry. Moreover, urology and dentistry with fewer numbers of clinical data in comparison with the above-mentioned areas, currently are in the path of translation (especially dentistry). In addition, otolaryngology and oncology with the lowest number showed more potential of research thorough understanding the properties that will help guiding the use of AM-derived products in these two areas in future. More than 50% of clinical studies were done or are developing in USA, which have the biggest share in market products. Subsequently, China, Egypt, India, Iran, and Germany with the ongoing clinical trials in different phases may have more approved products in near future.

1. Introduction

Regenerative medicine (RM), as a multidisciplinary area of translational medicine, contains tissue engineering, cell therapy, gene therapy, and the use of potentials of biomaterial and small molecules to cure disease. Amniotic membrane (AM) and amniotic fluid (AF), as valuable gifts from mothers that had been discarded after parturition, have a long history of application in different clinical areas. Low immunogenicity and anti-inflammatory effects of cytokine and growth factors via paracrine effect can also stimulate repair process of injured organs and diseases. Besides, nowadays, various products are distributed in market based on acellular amniotic membrane. AM after A-cellularization process can be used alone or by coating with different types of cells, which can be subsequently applied in tissue engineering or as a carrier for drug targeting. Moreover, AM- and AF-derived cells with considerable capabilities of differentiation into three germ layer, developed a new window toward cell- and gene-based therapies. Exosome, growth factors, mRNA, and conditioned medium derived AM are known as alternative options with no rejection concern to promote regeneration process in clinical approaches. Accumulating evidence showed that amnion, amniotic fluid, and cells derived from them are attractive materials in the field of regenerative medicine as a discarded source of fetal stem cells (Fig. 1A).

In this review, we aimed to list and then describe the most recent and relevant clinical and experimental data on the use of AM, fluid, and cells derived AM. The results of this review provide insight on developing new indication, cost-effectiveness, and clinical efficacy of AM derived products along with comprehensive overview of research status. For this purpose, the most important area of application was sorted and then

* Corresponding author. Department of Biochemistry, Erasmus University Medical Center, Rotterdam, the Netherlands.
** Corresponding author.
E-mail addresses: m.molapoursisakht@erasmusmc.nl, Mahsamollapur@yahoo.com (M.M. Sisakht).

https://doi.org/10.1016/j.placenta.2020.10.026
Received 26 September 2020; Received in revised form 18 October 2020; Accepted 21 October 2020
Available online 22 October 2020

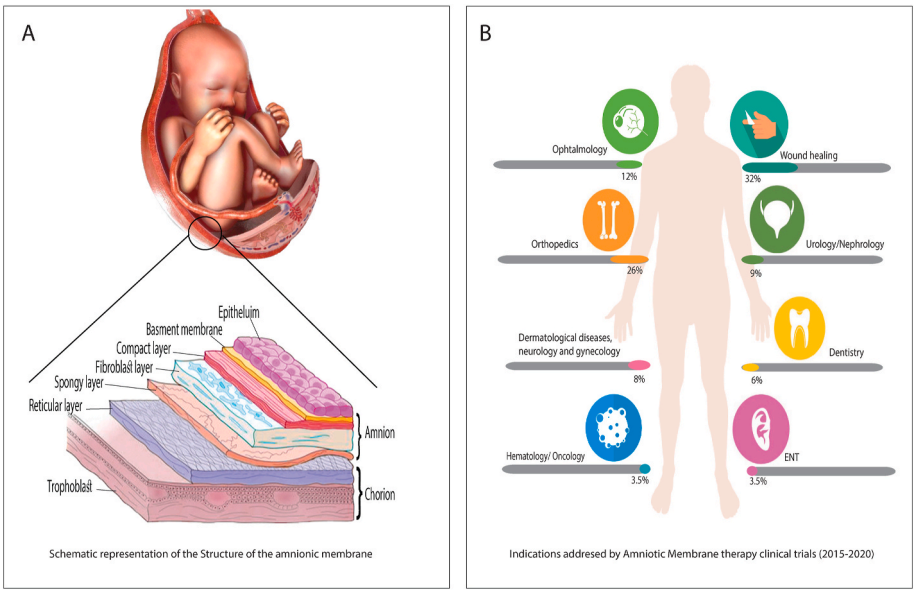

Fig. 1. Properties and potential use of amniotic membrane in regenerative medicine. A) Schematic representation of the structure of the amniotic membrane. B) Indication addressed by Amniotic Membrane therapy clinical trial from 2015 to 2020 (end of June).

considered for analysis as well as the assessment of the overall number of publications from 2015 to 2020. In this regard, it was revealed that dermatology (and more specific wound healing), orthopedic, ophthalmology, dentistry, urology, oncology, and otolaryngology more used AM compared to the other clinical areas (Fig. 1B).

For this purpose, all the studies reported in clinicaltrial.gov and NCBI clinical trial with special focus on the five last years were filtered using the following keywords: Amniotic membrane, Amniotic dressing, Amniotic membrane transplantation, and Amniotic membrane graft in two ways of alone and in conjunction with regenerative medicine, stem cells, tissue engineering, and cell therapy. After filtering, significant references were obtained from these databases and then designed based on clinical index. Afterward, the most important area of application was sorted and considered for analysis. The tables and figures presented in our paper are original. It should be noted, after performing statistical analysis on oncology and otolaryngology that have same share, we decided to discuss oncology as a more challengeable topic with the potential of more basic research at the end of discussion, along with five last categories.

2. Structure and biological properties of the hAM

Amniotic membrane (AM), as a translucent biological structure, is one of the thickest membrane (approximately 0.02–0.5 mm) in human body, and also is the inner layer of fetal membrane with no nerves, muscles or lymph vessels. Amniotic membrane similar to placenta, umbilical cord, and amniotic fluid is easily accessible and also considered as a valuable source of stem and progenitor cells. AM consists of amniotic epithelial cells (AEC), amniotic mesenchymal stem cells (AMSC), and fibroblasts. Moreover, AM stem cells have unique characteristics including anti-inflammatory, anti-fibrosis, anti-scarring, low immunogenicity, angiogenesis promotion, and oxidative stress inhibition [1,2]. in addition, AM can be divided into three layers as follows: 1. Epithelial monolayer, 2. Basement membrane, and 3. Hypocellular stromal matrix divided into compact, fibroblast, and spongy layers [2] which is show in Fig. 1A. Accordingly, the first layer, named as epithelial monolayer adjacent to the amniotic fluid, is constituted by a single homogeneous layer of cuboidal epithelial cells, which express epithelial specific markers including pan-cytokeratin (CK) 1, 2, 3, 4, 5, 6, 7, 8, 10, 13, 14, 15, 16, and 19; Carbohydrate Antigen 125; Mucin 16 [3–6]; EpCAM (CD326); E-Cadherin; and CD73 [7]. Furthermore, these cells can also synthesize and secrete extracellular matrix. The low immunogenicity of AM was provided by cells of this layer maybe due to a low expression of MCH II antigen [8]. The second layer, called as basement membrane, have large quantities of proteoglycans with heparin sulfate; collagen types I, III, IV, V, and VII; laminin; and fibronectin [9]. Correspondingly, this membrane contains lamina densa attached to the basal surface of AECs Integrins [10,11]. Finally, the third layer has a network of compact fibers made of collagen type I and III and small amounts of parallel bundles of collagen types II, IV, and V [12]. Afterward, during this process, fibroblast layer is composed of reticular fibers, fibroblasts, and large amounts of fibronectin and laminin, and AMSCs could also be isolated from this layer [13]. After the fibroblast layer, toward chorion, the spongy layer consist of proteoglycan and network of collagen fibers that separate the amnion from the chorion [14].

3. Clinical application of the hAM in RM: current situation

During the last decade, numerous studied reported the use of different physical shapes of AM with potential of accelerating regeneration under several clinical conditions.

The assessment of the overall number of the clinical trials and clinical trials publications performed between 2015 and 2020 revealed that dermatology diseases and wound healing have the biggest share with 35%, followed by orthopedic area with 23% share. Ophthalmology, urology/neurology, and dentistry with 12, 9, and 6% are in the second priority for research and development. Finally, otolaryngology and hematology/oncology with the same percentage (3.5%) showed more new features that need more studies (Fig. 1B). More than 50% of clinical studies are developing or were done in USA. Subsequently, China and Egypt between 10 and 20% and India, Iran and Germany have 2–5% of clinical trials, which may lead to products (Fig. 2). In following, we described the recent experimental advancement in five top clinical applications of AM, these fields are in the market or at least have potential to be in the market in up-coming years (with on-going late phase of clinical trials). Moreover, we discussed the oncology as one of the under development topic using AM to show the direction to future studies.

3.1. Dermatological diseases and wound healing

The first application of AM documented more than one hundred years ago was reported AM as biological wound dressing to speed up the process of healing in skin ulcer [15]. Immunomodulation, promotion in epithelization, anti-inflammatory, anti-angiogenetic, and anti-fibroblastic features could significantly promote healing of various kinds of wounds. The most well-known field of using AM that reviewed by different authors is tissue engineering for wound healing by AM [16]. During recent years, more innovative applications of AM to increase efficacy are also applied. Ghalei et al., in 2018 fabricated a bioactive wound dressing using electrospun silk fibroin and alginate hydrogel to deliver amniotic fluid to wound. Different ratios of Alginate and amniotic fluid studied and *In vitro* results showed that higher amounts of AF content could increase cellular proliferation as well as resulting in a higher collagen secretion [17]. 3D biodegradable scaffolds with potential to mimic the body's natural tissue were investigated to show the increased power of action such as pro-angiogenesis and cell migration of AM when combined with electrospun nanofiberous silk fibroin scaffold.

Accordingly, the results showed better mechanical characteristics, expression of angiogenesis, and vascular endothelial growth factors compared with amniotic membrane alone [18]. Accelerating formation of granulation tissue, increasing fibroblast proliferation, improving blood capillary formation, and developing collagen bundles were also shown to be resulted from the experiment examining chitosan/PVP gel combined with AM extract in burn wound healing [19,20]. Along with some of the above-mentioned aspects, combination of various cells [21] and AM derived scaffolds could also be promoted during recent years [22]. For instance; John et al., in 2019 developed Acellular AM as skin substitute, and AM was then prepared by detergent subjected to fibroblast and keratinocyte culture on chorion and epidermal sides, respectively [23]. The effect of human amniotic fluid stem cells on fibrotic scarring have also shown the increased wound healing process resembling fetal wound healing [24]. Zhou et al., in 2019 used decellular AM as a natural scaffold to seed the induced pluripotent stem cells (iPSC)--derived epithelial stem cells. This scaffold is substituted with the cultured cells which expressed CD200+/ITGA6+ Eps markers, reconstituted hair follicles after transplantation, and repaired skin defects in nude mice [25]. Another study published in 2018 as the first animal study was performed to evaluate the efficacy of menstrual blood-derived stem cell cultured on hAM. This study demonstrated a significant improvement in wound healing process compared with dried amniotic membrane-treatment [26].

In some studies, more novel active ingredient derived cells were also tested. Recently, urine stem cells derived from extracellular vesicle were also loaded in decellular AM, and showed the improved wound healing process in animal model [27]. Another study showed that exosomal MicroRNAs isolated from human amniotic epithelial cells accelerate wound healing process by paracrine mechanism promoting the proliferation and migration of fibroblasts [28]. Xiao et al., in 2019, expression vectors were applied on human amniotic mesenchymal stem cells to get a higher level of IL10. Accordingly, IL-10 is an anti-inflammatory and anti-fibrotic cytokine, which is essential for scar less wound healing processes. In this study, hAM MSCells revealed some synergistic effects such as modulating inflammation, increasing angiogenesis, and regulating ECM remodeling during wound healing [29].

Combination of some active biological materials was shown to have potential in accelerating wound healing. A study performed in 2017 showed that hyaluronic acid solubilized AM-treated wounds had regenerated skin more thicker, as well as more blood vessels and a greater number of proliferating keratinocytes [30]. It was indicated that the combination of AM with blood originated component could affect the activity of decellularized amnion. Kshersagar et al., in 2018 used AM activated with PRP and led to the increased cell migration, growth, and skin regeneration in burn model of their In vivo study [31]. Lyophilized AM in powder shape or in combination with natural gel-like biomaterial were examined for the wounds with irregular margins with various depth [32,33].

Accordingly, this type of process due to its easy application, no immune rejection, and decreased inflammation and oxidative stress [34,35] was considered by industry like lyophilized human amniotic membrane (dHAM) under brand name Amnioderm®, which was developed by PrimeCell Advanced Therapy Company to be used in cardiac surgery (sternum), gynecological diseases, abdominal surgery, ophthalmology, oncology, neurosurgery, orthopedics, and traumatology. Despite the fact that AM significantly speed up healing producer, Magnusson et al. in their study in 2017 performed an experiment to compare regenerative features of acellular fish skin grafts with acellular dHACM in severe battlefield injuries. The results have indicated that the fish skin graft possesses superior ability to stimulate 3-dimensional cell ingrowth compared to dHACM allograft. Correspondingly, this could be due to this fact that microstructure of acellular fish skin is highly porous [36] and have enough space for cells to attach. In addition, this result was also assessed in an enormous clinical study published in 2020 to compare the efficacy of the two therapeutic alternatives (dHACM and fish skin) in the treatment of chronic wounds. Wounds treated with fish skin have significantly healed faster than dHACM. It is also important to consider that dHACM-treated wounds were 76% more expensive compared to fish skin-treated wounds in this study [37]. Overall, these data in accordance with previous findings showed the significant effect of AM on wound healing process with cost-effectiveness. Besides, there are few reports performed under the dermatological conditions such as erythema, hyperkeratosis, and scar using the irradiated AM gel, which showed the potential of using AM in the different aspects of dermatology [38].

During last decade, studies showed that platelet gel (PG) or platelet rich plasma (PRP gel) also can promoting regeneration through induction of cell differentiation and proliferation, extracellular matrix formation, migration and recruitment of other cells to the site of injury and by increasing collagen production [39]. Both treatment with AM and PLT/PRP gel provoke therapeutic action by wide range of GFs. It would be expected for both sources to perform similarly due to the wide range of similarities between the mechanisms of action (Paracrine effect) and the associated growth factor profiles [40]. PG from cord blood (CBPG) as a new source of concentrated platelet, also supporting tissue repair and regeneration, angiogenesis and proliferative properties, considerably [41]. In addition, CBPG have a high clinical potential in post-oral and maxillofacial surgery [42].

In some studies, researchers try to combine this two method to increase efficacy of treatment, for instance; autologous PRP gel (Eye-PRP clot) used in conjunction with AM to treat corneal conditions [43,44]. Mixture of Amniotic fluid-derived stem cells with PRP, used for alveolar bone tissue engineering strategy for alveolar defect restoration by Wang et al., in 2017 [45]. Moreover, platelet poor plasma (PPP) gel combined with amnion (PPPA) showed the improved level of GF expression for further use in re-epithelialization and wound healing process, study by Yang et al., in 2017 showed up-regulated of paracrine molecules such as HGF, TGFβ, VEGF, IGF-1 and KGF in human umbilical cord-derived mesenchymal stem cells embedded in PPP gel combined with AM, in compare with monolayer culture of human MSC [46]. Data from preclinical and clinical studies showed that wound healing [47,48] and sport medicine [49] are most important area which used PRP and PLT gel. However, both treatments have same primary methods of action on target [50,51], but production of active PRP depends significantly on the standard operating procedures and severely affected by donor-based biological parameter. Additionally, growth factor profile of AM, especially, amnion-derived fluid is more consistent. In total, we can say combination therapy by using AM as a scaffold or PRP enrichment by AM/AF could be an option to strongly promote regeneration.

Table 1
List of Clinical trials of regenerative medicine and Amniotic membrane.

Study title	Conditions	Interventions	Phase	Field of study	age	Number Enrolled	Study Start	country	Status	ID
Comparison of Effectiveness of Amniotic Membrane and Duoderm Dressings in Pediatric Burns	2nd and 3rd degree fresh burn	amniotic membrane dressing	Phase 2/3	wound healing	12-Jan	172	2019	Pakistan	Recruiting	NCT04030754
Human Amniotic Membrane Plug for Large Macular Holes	Macular holes	amniotic membrane plug with vitrectomy	N/A	Ophthalmology	Older than 5	10	2019	Egypt	Recruiting	NCT03917602
Safety and Clinical Outcomes With Amniotic and Umbilical Cord Tissue Therapy for Numerous Medical Conditions	many	Amniotic tissue Procedure	Phase1	many	Older than 18	5000	2019	USA	Recruiting	NCT03899298
Amniotic Umbilical Cord Particulate During Total Knee Arthroplasty	Osteoarthritis: Joint Replacement Surgery	CLARIX FLO	N/A	orthopedics	older than18	20	2019	USA	Recruiting	NCT03912116
Prospective Study on Implantation of Amniotic Membrane (AM) Over Pancreatic Anastomosis After Pancreaticoduodenenctomy: Efficacy on Preventing Clinically Relevant Pancreatic Fistula (POPF)	Post Operative Pancreatic Fistula/ Pancreatic Resection	Amniotic Membrane implantation	Early phase 1	surgery	18–85	20	2019	Italy	Recruiting	NCT03891225
Application of Dried Human Amnion Graft to Improve Postprostatectomy Incontinence and Potency	Continence Potency Complication Biochemical Recurrence	Dehydrated amniotic membrane	Phase 2/3	Urology	40–80	328	2019	Germany	Not yet recruiting	NCT03864939
Addition of Dehydrated Human Amnion-Chorion Membrane During Scaling and Root Planing	Periodontal Diseases	BioXclude™	N/A	dentistry	18–100	250	2019	USA	Not yet recruiting	NCT03836378
Amnion-Based Injections in the Shoulder	Osteoarthritis of the Shoulder Adhesive Capsulitis	Amnion Injection and Betamethasone injection	N/A	orthopedics	18–100	80	2018	USA	Not yet recruiting	NCT03770546
Human Amniotic Epithelial Cells Prevent Acute Graft-versus-host Disease After Hematopoietic Stem Cell Transplantation (hAECs-GVHD)	Acute-graft-versus-host Disease	human amniotic epithelial cells	N/A	Hematology	18–70	27	2018	china	Not yet recruiting	NCT03764228
Corticosteroid vs. Amniotic Fluid Injections in Patients With Trigger Finger	Stenosing Tenosynovitis	Amniotic fluid injection	N/A	Orthopedics	older than 18	100	2018	USA	Enrolling by invitation	NCT03583151
hAECs Are Preliminarily Applied in Allogeneic Hematopoietic Stem Cell Transplantation	Leukemia	human amniotic epithelial cells	N/A	Hematology	all	30	2018	china	Recruiting	NCT03759899
Amnion Wound Covering for Enhanced Wound Healing	Burns-Wound of Skin	Amnion Membrane Powder	phase 1	wound healing	18–65	10	2018	USA	Not yet recruiting	NCT03754218
Efficacy of Amniotic Membranes in Complex Genitourinary Reconstruction	urinary fistula-Hypospadias		N/A	urology	6month-99years old	0	2018	USA	Withdrawn	NCT03685955
Comparison of Platelet Rich Fibrin as Graft & Membrane Vs Hyaluronic Acid & Amniotic Membrane in the Treatment of Interradicular Defects (CPRFGMHATID)	Furcation Defects	Conventional Flap surgery	N/A	dentistry	30–50	14	2018	india	completed	NCT03578744
Comparison Tympanoplasty With Membrane Amniotic and Autologous Fascia	Tympanic Membrane Perforation	Tympanoplasty With Autologous Fascia	N/A	Otorhinolaryngologic	all	30	2018	iran	completed	NCT03569969
A Case Series to Investigate the Safety and Efficacy of Weekly Application of Dehydrated Human Amnion/ Chorion Membrane in the Treatment of Pressure Ulcers	Pressure Ulcer	Dehydrated Human Amnion/Chorion Membrane	N/A	wound healing	older than 18	20	2018	usa	Enrolling by invitation	NCT03529578
Amniotic Membrane for Recurrent Macular Hole	Macula Hole Retinal Detachment	amniotic membrane graft	N/A	Ophthalmology	all	10	2018	Egypt	Recruiting	NCT03528122
Limb Salvage Through Tissue Engineering: A Novel Treatment Modality Using Dehydrated Human Amnion/Chorion Membrane	wounds and Soft Tissue Injuries	Dehydrated Human Amnion/Chorion Membrane	N/A	wound healing	older than 18	53	2018	usa	recruiting	NCT03521258
Evaluation of Outcomes With Amniotic Fluid for Musculoskeletal Conditions Musculoskeletal Conditions	Osteoarthritis-Tendinitis	Amniotic Fluid Tissue Product	phase 2/3	Orthopedics	older than 18	100	2018	USA	recruiting	NCT03390920
	Corneal Perforation		N/A	Ophthalmology	all	6	2018	Egypt		NCT03500796

(*continued on next page*)

Table 1 (*continued*)

Study title	Conditions	Interventions	Phase	Field of study	age	Number Enrolled	Study Start	country	Status	ID
Combined Amniotic Membrane and Platelet Rich Plasma Clot for Management of Central Corneal Perforation		amniotic membrane and platelet rich plasma clot							Not yet recruiting	
Micronized dHACM vs. Saline in the Treatment of Osteoarthritis of the Knee	Knee Osteoarthritis	Micronized Dehydrated Human Amnion/Chorion Membrane	Phase 2	Orthopedics	21–80	318	2018	usa	recruiting	NCT03485157
Use of Human Dehydrated Amnion Chorion Allograft in Laryngectomy/Pharyngectomy	Larynx Cancer-Pharynx Cancer	Dehydrated human amnion/chorion membrane	N/A	oncology	Older than 18	80	2018	usa	Enrolling by invitation	NCT03479463
Long Term Results of Amniotic Membrane Transplant in Bullous Keratopathy Patients	Bullous Keratopathy	Amniotic membrane transplant	N/A	wound healing	all	27	2018	Hong Kong	completed	NCT03450954
Corticosteroid vs. Amniotic Fluid Injections in Patients With Trigger Finger	Stenosing Tenosynovitis	Amniotic fluid injection	phase 3	Orthopedics	older than 18	100	2018	USA	enrolling by invitation	NCT03583151
Safety & Efficacy FloGraft (Micronized Human Amnion Chorion Membrane)® in Adults With Pain Due to OA of the Knee (OA)	Osteoarthritis	Micronized Human Amnion Chorion Membrane	phase2	orthopedics	older than 30	320	2018	usa	Not yet recruiting	NCT03441607
Using of Irradiated Amnion Dressing for the Treatment of Skin Ulcer	ulcers	dried human amniotic membrane sterilized by gamma irradiation	N/A	wound healing	all	3	2016	Egypt	completed	NCT03440528
Human Amniotic Epithelial Cell in Treatment of Refractory Severe Intrauterine Adhesion	Intrauterine Adhesion	human amniotic epithelial cells	phase 1	Urology	20–45	20	2018	china	Not yet recruiting	NCT03381807
Preliminary Investigation of ViaShield™ Amnion Patch as an Anti-Adhesive Barrier in Hemicraniectomies (ViaShield)	Hemicraniectomies	ViaShield™ amnion patch	N/A	neurosurgery	Older than 18	0	2018	usa	Withdrawn	NCT03371316
Effect of Implanting Allogenic Cytokines Derived From Human Amniotic Membrane (HAM) and Mesenchymal Stem Cells Derived From Human Umbilical Cord Wharton's Jelly (HUMCWJ) on Pain and Functioning of Knee Osteoarthritis	Osteo Arthritis-Musculoskeletal Disease	allogenic cytokines derived from human amniotic membranes	N/A	Orthopedics	50–85	60	2017	usa	active,not recruiting	NCT03337243
Use of Human Dehydrated Amnion/Chorion (DHACM) Allograft in Partial Nephrectomy	Partial Nephrectomy-Kidney Cancer	Dehydrated human amnion/chorion membrane	phase 4	nephrology	older than 18	61	2017	usa	completed	NCT03323021
Bio ACL Reconstruction Amnion Collagen Matrix Wrap and Stem Cells (Bio ACL RCT)	Anterior Cruciate Ligament Rupture	Collagen based -membrane derived from amniotic tissue	N/A	orthopedics	18–45	40	2017	usa	active,not recruiting	NCT03294759
Efficacy of an Amniotic Fluid Derived Allograft, (FlōGraft®) in Rotator Cuff Repairs: A Prospective Study	Rotator Cuff Tear	human amniotic fluid derived allograft	N/A	Orthopedics	all	260	2017	USA	recruiting	NCT03379324
Study for the Treatment of Ocular Chronic Graft-Versus-Host Disease (GVHD) With Amniotic Fluid Eye Drops (AFED)	Ocular Graft Versus Host Disease	Amniotic Fluid Eye Drops	phase 2	ophtalmology	older than 18	15	2017	USA	Not yet recruiting	NCT03298815
Uses of Irradiated Human Amniotic Membrane in the Treatment of Dystrophic Epidermolysis Bullosa Patients	Epidermolysis Bullosa chronic wound	dried human amniotic membrane	N/A	dermatology	12′-45	8	2017	Egypt	completed	NCT03942250
Human Amniotic Epithelial Cells for Asherman's Syndrome	Asherman's Syndrome	human amniotic epithelial cells	phase 1	gynecology	20–40	50	2017	china	Not yet recruiting	NCT03223454
Evaluation of Outcomes With Amniotic Fluid for Musculoskeletal Conditions Musculoskeletal Conditions	musculoskeletal conditions	Amniotic Fluid Tissue Product	phase2/3	Orthopedics	older than 18	200	2017	USA	recruiting	NCT03390920
Affinity Prospective Diabetic Foot Trial Crossover Group	Diabetic Foot Ulcer	human amniotic membrane graft	N/A	wound healing	older than 18	20	2017	usa	recruiting	NCT03205436
Effect of Human Amniotic Epithelial Cells on Children With Spastic Cerebral Palsy	Spastic Cerebral Palsy	human amniotic epithelial cells	phase 1	neurology	1–5	10	2017	china	Unknown	NCT03107975

(*continued on next page*)

Table 1 (*continued*)

Study title	Conditions	Interventions	Phase	Field of study	age	Number Enrolled	Study Start	country	Status	ID
Amnion-Chorion Allograft Barrier Used for Root Surface and Guided Tissue Regeneration for Periodontal Intrabony Defects and Grade II Furcation Defects.	Periodontal Diseases	Amnion-Chorion allograft membrane	phase 4	dentistry	18–75	20	2017	usa	recruiting	NCT03095976
Protocol for the Clinical Evaluation of Lyophilized Amniotic Fluid in the Treatment of Knee Osteoarthritis	Osteoarthritis	Amniotic Fluid Injection	N/A	Orthopedics	older than 30	180	2017	USA	Unknown	NCT03074526
ReNu™ Marrow Stimulation Augmentation	Osteochondral Defect	ReNu™ allograft	N/A	Orthopedics	18–55	8	2017	usa	recruiting	NCT03036878
Treatment of Nonunion of Limb Fracture With Human Amniotic Epithelial Cells (hAECs)	Nonunion Fracture	Human Amniotic Epithelial Cells	phase 1/2	Orthopedics	18–80	36	2017	china	Not yet recruiting	NCT03031509
Hearing Results and Post Surgical Outcomes Using Amniotic Membrane Allograft vs. Fascia for Tympanoplasty	Tympanic Membrane Perforation	Skye Barrier(Amniotic membrane allograft)	N/A	Otorhinolaryngologic	older than 3	50	2017	usa	Enrolling by invitation	NCT03028272
Amniotic Membrane Wrapping and Tenolysis Versus Tenolysis Alone for Treatment of Tendon Adhesions of the Hand/Wrist	Tendinopathy	Amnion Tendon Wrapping	N/A	Orthopedics	older than 18	40	2017	usa	Unknown	NCT03013582
Amniotic Membrane for Donor Site Healing	burns	Amniotic membrane dressing	N/A	wound healing	older than 18	4	2017	usa	completed	NCT02947737
Amniotic Membrane Allograft Application in the Management of Venous Leg Ulcerations (AmnioExCel)	Varicose Ulcer-Venous Insufficiency	AmnioExCel dressing	N/A	wound healing	older than 18	40	2017	usa	enrolling by invitation	NCT02929056
A Therapeutic Trial of Human Amniotic Epithelial Cells Transplantation for Primary Ovarian Insufficiency Patients (POI)	Primary Ovarian Insufficiency	human amniotic epithelial cells transplantation	phase 1	gynecology	20–39	2	2016	china	Unknown	NCT02912104
Human Amniotic Versus Synthetic Membrane as a Transient Skin Cover for Pediatric Burns	Burns	Amniotic Membrane Dressing	N/A	wound healing	up to 15 years old	60	2016	Chile	recruiting	NCT02904941
Evaluation of Amniotic Fluid Product in Knee Osteoarthritis	Knee Osteoarthritis	Amniotic Fluid Injection	N/A	Orthopedics	older than 30	60	2016	USA	Completed	NCT02768155
Effect of Fresh Amniotic Membrane in the Treatment of Diabetic Foot Ulcers	Diabetic Foot Ulcers	human amniotic membrane graft	N/A	wound healing	older than 18	89	2016	usa	completed	NCT02880592
Human Amniotic Epithelial Cells for Treatment of Bronchial Fistula	Bronchial Fistula	human amniotic epithelial cells	N/A	surgery	18–75	10	2016	china	Unknown	NCT02959333
Prospective, Comparitive, Randomized Study of Allograft Versus Skin Substitute in Non-healing Diabetic Foot Ulcers	Diabetic Foot Ulcer	Amnionic Membrane Graft	N/A	wound healing	older than 18	60	2016	usa	recruiting	NCT02870816
Case Series of Weekly Applications of dHACM in Treatment of Pressure Ulcers	Pressure Ulcer	dehydrated human amnion/chorion membrane	N/A	wound healing	older than 16	10	2016	usa	completed	NCT02861560
ReNu™ vs. Corticosteroids for the Treatment of Plantar Fasciitis	plantar fasciitis	ReNu Injection	N/A	Orthopedics	18–75	150	2016	usa	recruiting	NCT02982226
Dehydrated Human Amnion Chorion Membrane (dHACM) vs. Control in the Treatment of Partial Thickness Burns.	Partial Thickness Burns	Dehydrated Human Amnion/Chorion Membrane	phase 1/2	wound healing	older than 1 year old	60	2016	usa	recruiting	NCT02765737
Miami Membrane for Potency (MMEP) Trial (MMEP)	Prostate Cancer	Amniotic Membrane Placement	phase 2	Urology	40–80	24	2016	usa	terminated	NCT02710422
Randomized AmnioFix Study During Radical Prostatectomy	Prostate Cancer	dehydrated human amnion/chorion membrane	phase 2	Urology	35–75	0	2016	usa	Withdrawn	NCT02645591
Uses of Gamma Irradiated Amniotic Membrane as an Alternative Method in Psoriasis Treatment	Psoriasis	human amniotic membrane extra-cellular matrix as a topical treatment	N/A	dermatology	20–40	22	2015	egypt	completed	NCT03440541
Prevention of Amputation in Diabetic Foot Ulcers Using Amniotic Tissue	Diabetic Foot Ulcer	AMNIOEXCEL®	N/A	wound healing	older than 18	20	2015	usa	active,not recruiting	NCT02579993

(*continued on next page*)

Table 1 (*continued*)

Study title	Conditions	Interventions	Phase	Field of study	age	Number Enrolled	Study Start	country	Status	ID
Study to Evaluate the Safety and Efficacy of CHAM* for the Treatment of Diabetic Foot Ulcers (*OTI-15-01)	Chronic Diabetic Foot Ulcers	Cryopreserved Human Amniotic Membrane	phase 3	wound healing	18–80	108	2015	usa	terminated	NCT02571738
Evaluation of Amnion Chorion Membrane and Demineralized Bone Matrix Putty Allograft in Periodontal Intrabony Defects	Chronic Periodontitis	Amnion chorion membrane	N/A	dentistry	35–50	22	2015	Egypt	completed	NCT02679209
Biodegradable Synthetic Scaffold as a Substitute for hAM in Limbal Epithelial Cells Transplant in LSCD Patients	Limbal Stem Cell Deficiency	human amniotic membrane	N/A	Ophtalmology	18–70	5	2015	India	completed	NCT02568527
DHACM in Robotic Assisted Laparoscopic Prostatectomy (RALP)	Erectile Dysfunction	dehydrated human amnion/chorion membrane		Urology	40–70	230	2015	usa	Unknown	NCT02526173
Pilot Study to Evaluate Clinical Outcomes With the Use of Biovance Following Keloid Scar Revision Surgery	Keloid Scar	decellularized, dehydrated human amniotic membrane	phase 4	dermatology	21–80	10	2015	USA	Unknown	NCT02521402
Postmarket Study to Evaluate Biovance® in Diabetic Foot Ulcers	Diabetic Foot Ulcers	Dehydrated Decellularized Human Amniotic Membrane Allograft	N/A	wound healing	older than 18	51	2015	USA	Unknown	NCT02506452
Autologous Oral Mucosa Transplantation for Limbal Stem Cell Deficiency	Limbal Stem-cell Deficiency	amniotic membrane transplantation	N/A	Ophtalmology	older than 18	27	2015	Germany	completed	NCT03226015
NuShield/Affinity for the Treatment of Neuropathic Diabetic Foot Ulcers	diabetic foot ulcers	NuShield	N/A	wound healing	older than 19	96	2015	USA	completed	NCT02461641
Study of Amniotic Membrane Graft in the Management of Diabetic Foot Ulcers	diabetic foot ulcers	human amniotic membrane	N/A	wound healing	older than 18	40	2015	USA	Unknown	NCT02399826
Healing of Persistent Epithelial Defects	persistent corneal epithelial defects	Ambiodisk amniotic membrane-Prokera amniotic membrane	N/A	Ophtalmology	older than 18	0	2015	USA	Withdrawn	NCT02395952
A Prospective Study of NuCel® in Cervical Spine Fusion	diseases of the cervical spine.	NuCel® allograft	N/A	Orthopedics	older than 21	61	2015	USA	terminated	NCT02381067
Study of ST266 Eye Drops in Treating Dry Eye	Dry Eye Syndrome	Amnion-derived Cellular Cytokine Solution (ACCS) Eye Drops	phase 1	Ophtalmology	older than 18	30	2015	USA	completed	NCT02369861
The Effect of Human Amniotic Membrane Allograft on Functional Recovery After Flexor Tendon Repair	Flexor Tendon	amnionic membrane allograft	N/A	Orthopedics	older than 18	5	2015	Finland	terminated	NCT02361814
A Comparative Study of the Healing of Chronic Ulcers of Recessive Epidermolysis Bullosa: Dressing vs Amniotic Membrane (MABUL)	Epidermolysis Bullosa	amniotic membrane dressing	phase 3	dermatology	2′-80	22	2015	France	recruiting	NCT02286427
Amnion Membrane as a Novel Barrier in the Treatment of Intrabony Defects: A Controlled Clinical Trial	intrabony periodontal defects	amniotic membrane	N/A	dentistry	35–55	10	2015	Iran	Complete	26009915
Sutureless fixation of amniotic membrane for therapy of ocular surface disorders.	Ocular Surface Disorders	amniotic membrane transplantation	N/A	ophthalmology	24–85	7	2015	USA	Complete	25955359
Efficacy of Autologous Melanocyte Transplantation on Amniotic Membrane in Patients With Stable Leukoderma: A Randomized Clinical Trial	Vitiligo	amniotic membrane (AM)–cultured epidermal cell grafting	N/A	Dermatology	18–57	24	2015	Spain	Complete	25902042
Trabeculectomy With Mitomycin-C Versus Trabeculectomy With Amniotic Membrane	bilateral primary open-angle glaucoma	amniotic membrane transplant	N/A	ophthalmology	older than 60	52	2015	Egypt	Complete	24844538

(*continued on next page*)

Table 1 (*continued*)

Study title	Conditions	Interventions	Phase	Field of study	age	Number Enrolled	Study Start	country	Status	ID
Transplant: A Medium-term Randomized, Controlled Trial.										
Dehydrated human amnion/chorion membrane treatment of venous leg ulcers: correlation between 4-week and 24-week outcomes.	venous leg ulcers	dehydrated human amnion/								
chorion membrane allograf	N/A	wound healing	ND	44	2015	UK				
A prospective, randomized, controlled, multi-centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers.	chronic lower extremity diabetic ulcers	dehydrated human amnion/chorion membrane allograft	N/A	wound healing	older than 18	60				
Maximizing the safety of glycerol preserved human amniotic membrane as a biological dressing.	burns	human amniotic membrane	N/A	wound healing	10 ′-50	40				
Clinical evaluation of amniontic products after transcervical resection of intensive degree of intrauterine adhesions	intrauterine adhesions	dehydrated human amnion/chorion membrane allograft	N/A	urology	???	57				
Biological Amnion Prevents Flexor Tendon Adhesion in Zone II:										
A Controlled, Multicentre Clinical Trial	Tendon adhesion	amniotic membrane transplantation	N/A	Orthopedics	21–65	89				
The effects of amniotic membrane stem cell-conditioned medium on photoaging.	aging	amniotic membrane stem cell								
conditioned medium	N/A	dermatology	45–55	24	2018	Ind				
Application of amniotic membrane for covering mastoid cavity in canal wall down mastoidectomy.	chronic suppurative otitis media with cholesteatom	Amniotic membranes graft	N/A	Otorhinolaryngology	20–73	148				
Clinical and radiographic evaluation of amnion chorion										
membrane and demineralized bone matrix putty allograft										
for management of periodontal intrabony defects:										
a randomized clinical trial	periodontal									
intrabony defects	amnion chorion									
membrane	N/A	dentistry	32–49	22	2018	Egy				
A confirmatory study on the efficacy of dehydrated human amnion/chorion membrane dHACM allograft in the management of diabetic foot ulcers: A prospective, multicentre, randomized, controlled study of 110 patients from 14 wound clinics	diabetic foot ulcers	dehydrated human amnion/chorion								
membrane	N/A	wound healing	older than 65	110	2018	US/				
Use of an aseptically processed, dehydrated human amnion and chorion membrane improves likelihood and rate of healing in chronic diabetic foot ulcers: A prospective, randomized, multi-centre clinical trial in 80 patients.	chronic diabetic foot ulcers	human amnion and chorion allograft	N/A	wound healing	older than 18	80				

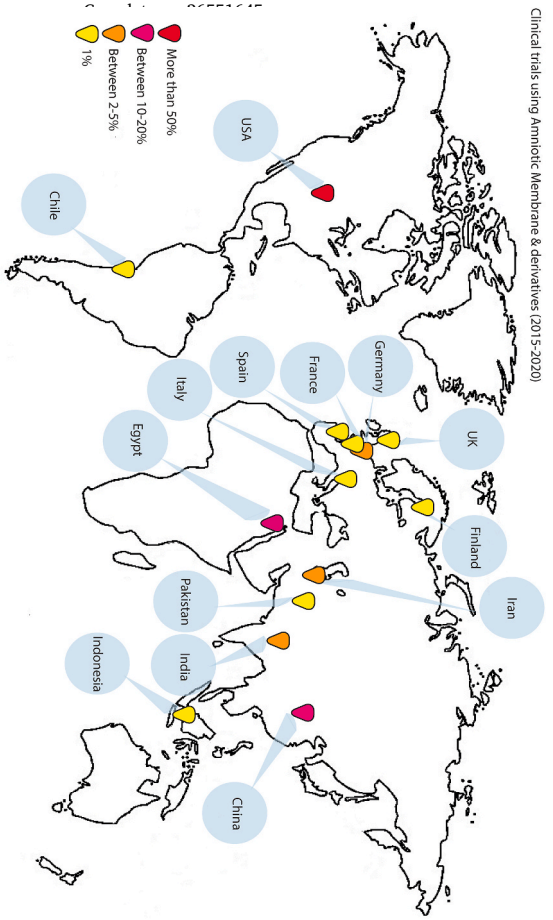

Fig. 2. Clinical trials using Amniotic membrane and derivatives around the world from 2015 to 2020 (end of June).

Table 2
List of Amniotic membrane and derivatives products under development or available in the market.

Commercial name	Product details	Clinical or potential clinical application	Company	Reference
AmnioBand® Membrane	Dehydrated allograft placental matrix	Wound covering	MTF Biologics	https://www.mtfbiologics.org/
Biovance®	Decellularized, dehydrated human amniotic membrane (DDHAM)	Wound covering	BIOVANCE	https://www.biovance.net/
Epifix®	Dehydrated Human Amnion/Chorion Membrane (dHACM) Allograft	Acute and chronic wounds	Mimedx	https://mimedx.com
AmnioFix/ PURION®	Dehydrated Human Amnion/Chorion Membrane (dHACM) Allograft	Acute and chronic wounds	Mimedx	https://mimedx.com
EpiCord®	Dehydrated Human Umbilical Cord Allograft	Acute and chronic wounds	Mimedx	https://mimedx.com
EpiBurn	Dehydrated Human Amnion/Chorion Membrane (dHACM) Allograft	Acute and chronic wounds	Mimedx	https://mimedx.com
AmnioCord	Thick membrane derived from umbilical cord	Acute and chronic wounds	Mimedx	https://mimedx.com
AmnioFill	Non-viable cellular placental tissue matrix allograft	Acute and chronic wounds	Mimedx	https://mimedx.com
GrafixCore™ (Grafix® PL Core)	Cryopreserved placental membrane	Diabetic foot ulcers, Burns, Arterical ulcers, Deep tunneling wounds, Pressure ulcers, Pyoderma gangrenosum, Surgical dehiscence Trauma, Surgical incisions, Venous leg ulcers	Osiris	http://www.osiris.com/
GrafixPrime™ (Grafix ® PL Prime)	lyopreserved placental amniotic membrane	Acute and chronic wounds	Osiris	http://www.osiris.com/
NuCel®	Cryopreserved amniotic suspension allograft	Bone Fusion, Tendon Repair, Acute Limb Salvage, Acute Wounds & Burns	organogenesis	https://organogenesis.com/pdf/B-201-Product-Portfolio-Brochure.pdf
Affinity®	fresh, hypothermically stored amniotic membrane	Tendon Repair, Cartilage & Osteochondral Defects, Acute & Chronic Wounds	organogenesis	https://organogenesis.com/pdf/B-201-Product-Portfolio-Brochure.pdf
NuShield®	Dehydrated amnion-chorion membrane	Tendon Repair, Spine Adhesions & Fibrosis, Acute & Chronic Wounds	organogenesis	https://organogenesis.com/pdf/B-201-Product-Portfolio-Brochure.pdf
ReNu®	Cryopreserved amniotic suspension allograft	Osteoarthritis, Tendinopathy, Plantar Fasciitis	organogenesis	https://organogenesis.com/pdf/B-201-Product-Portfolio-Brochure.pdf
ActiveBarrier®	membranes are sterile allografts	Acute and chronic wounds	Skye Biologics	https://www.skyebiologics.com/
WoundEx® Membrane	Fast Acting® dehydrated amniotic membrane	Acute and chronic wounds	Skye Biologics	https://www.skyebiologics.com/
WoundEx® Flow	Flowable human placental connective tissue matrix skin substitute	Acute and chronic wounds	Skye Biologics	https://www.skyebiologics.com/
OculoMatrix® & VisiDisc®	FastActing® amniotic membrane allografts available in a thin, amnion-only, and a thick, chorion-based configuration	Ophthalmology	Skye Biologics	https://www.skyebiologics.com/
CryoMatrix® & ActiveMatrix®	Flowable allografts derived from a complete placental source, not just a membrane particulate, or amniotic fluid	Surgical use	Skye Biologics	https://www.skyebiologics.com/
BurnMatrix®	Highly concentrated placental tissue matrix allograft	Burn Treatment	Skye Biologics	https://www.skyebiologics.com/
WoundEx® Membrane	FastActing® dehydrated amniotic membrane skin substitute	Chorion-based Wound	Skye Biologics	https://www.skyebiologics.com/
ViaShield®	Dual Layer Amnion Patch processed from human placental tissue	Chronic wound	Globus medical	https://www.globusmedical.com/
CLARIX FLO	Umbilical cord based injectable	Sports Medicine	Amniox medical	https://amnioxmedical.com/clarix-injectable/
BioXclude®	Dehydrated human deepithelialized amnion-chorion membrane	Variety of dental, endodontic, oral maxillofacial, and periodontal regenerative procedures	Snoasis Medical	https://www.snoasismedical.com/meet-bioxclude
NEOX	Cryopreserved Umbilical Cord and Amniotic Membrane Matrix	Wound	Amniox	https://amnioxmedical.com/
CLARIX CORD	Cryopreserved Umbilical Cord + Amniotic Membrane Matrix	Wound	Amniox	https://amnioxmedical.com/
CLARIX FLO	Umbilical cord based injectable	Sports Medicine	Amniox	https://amnioxmedical.com/
BioDFactor®	Cryopreserved allograft derived from the human placental tissues	Tissue voids and defects Localized areas of inflammation	Encompass Biologics	https://www.encompassbiologics.com/
	Adhesion barrier allograft derived from the human amnion	Dura protection, Interspinal muscle protection, Laminectomy,	Encompass Biologics	https://www.encompassbiologics.com/

(continued on next page)

Table 2 (*continued*)

Commercial name	Product details	Clinical or potential clinical application	Company	Reference
BioDDryFlex® & BioDFence G3®		Craniotomy, Nerve bundle protection, Microdiscectomy Discectomy		
BioDRestore™	Flowable tissue allograft derived from amniotic tissues	Wound covering	Encompass Biologics	https://www.encompassbiologics.com/
Cygnus®	Family of amnion patch allografts	Spine & Neurosurgery, Foot & Ankle, Wound Care, Burn Care, Dermatology, Ophthalmology, Oral Surgery	VIVEX Biologics	https://vivex.com/
AlloGen®	Liquid matrix allograft derived from amniotic fluid	Sports Medicine	VIVEX Biologics	https://vivex.com/
Amnioexel	human placental-based tissue	Wound	Integra	https://www.integralife.com/
AmnioMatrix®	Cryopreserved suspension allograft derived from the amniotic membrane and components of the amniotic fluid	Wound	Integra	https://www.integralife.com/
BioDOptix®	Amniotic Extracellular Matrix is a dehydrated, membrane allograft derived from human amniotic	Ocular tissue repair	Integra	https://www.integralife.com/
BioDDryFlex® & BioD	Amniotic Tissue Membrane	Surgical Reconstruction, Sports Medicine	Integra	https://www.integralife.com/
AmbioDisk™	Amniotic Membrane Graft	• Non-Healing Epithelial Defects, Neurotrophic Ulcerations, Corneal Erosions, Acute Chemical/Thermal Burns, Post-Infectious Keratitis (herpetic, vernal, bacterial)	IOP Ophthalmics	https://www.ophthalmologyweb.com/
R3 Stem Cell	Amnion Derived Stem Cell Activator Injections	Orthopedic, Neurologic, Urologic, Autoimmune, Renal, Cardiac and Pulmonary Condition	R3 Stem Cell	https://r3stemcell.com/
FlōGraft®	Injectable amniotic fluid-derived allograft	Wound	Applied Biologics	https://www.appliedbiologics.com/
XWRAP®	Chorion-free amniotic membrane Allograft	Wound	Applied Biologics	https://www.appliedbiologics.com/
AmnioELITE™	Liquid Amniotic Fluid Allograft	Sports Medicine	Surgilogix	http://www.surgilogix.com/
SXBarrier™	Human amniotic membrane allograft	Covers wounds and supports native tissue	Surgilogix	http://www.surgilogix.com/
SXFluid™	Amniotic Fluid Allograft	Wound and surgical setting	Surgilogix	http://www.surgilogix.com/
PROKERA®	cryopreserved amniotic membrane	ocular conditions such as keratitis, moderate to severe dry eye disease, recurrent corneal erosions, filamentary keratitis, persistent epithelial defects, neurotrophic corneas, herpetic ulcers, and many other ocular surface diseases such as chemical burns and Stevens-Johnson syndrome	Biotissue tech company	https://www.biotissue.com/
AmnioGraft	cryopreserved amniotic membrane	Corneal ulcers, pterygium, mechanical dry eye (also known as conjunctivochalasis), excision of tumors, chemical burns, and Stevens-Johnson Syndrome.	Biotissue tech company	https://www.biotissue.com/
AmnioGuard	Umbilical cord graft	Ophtalmology	Biotissue tech company	https://www.biotissue.com/
AMNIODERM®	Amniotic membrane lyophilized	wound	Amnio derm	https://www.amnioderm.com
AMNIOCHORION®	lyophilized human amniochorion membrane (dHACM)	wound	Amnio derm	https://www.amnioderm.com
AMNIOGEL®	Micronized amniotic or amniochorion membrane prepared for administration either in form of gel	aesthetic medicine, scars healing after a surgical procedures (e.g. Caesarean section), wound, reduces scarring	Amnio derm	https://www.amnioderm.com
AMNIOFLUID®	Micronized amniotic or amniochorion membrane	Sport Medicine	Amnio derm	https://www.amnioderm.com
AmiCare R	dried amniotic membrane	Wound covering	Royan Stem Cell Technology Company	https://www.rsct.ir/En
OcuReg-A R	acellular amniotic membrane	Wound covering	Royan Stem Cell Technology Company	https://www.rsct.ir/En
OcuReg-C R	cryopreserved amniotic membrane	Wound covering	Royan Stem Cell Technology Company	https://www.rsct.ir/En
OcuReg-L R	lyophilized amniotic membrane	Wound covering	Royan Stem Cell Technology Company	https://www.rsct.ir/En

3.2. Orthopedic clinical condition

AM, decullarized AM, cells isolated from AM or AF, and cells cultured on AM have been evaluated for the repair of tendon and ligament, attenuation of cartilage and joint space diseases, prevention of scarring and adhesion formation in spinal fusion procedures. More recently, potential of hAMSCs differentiation to osteogenic cells, which were showed by presenting phenotype markers [52]. However, hAM showed no considerable osteogenic differentiation in *in vitro* study [53]. Osteogenic differentiation of hAMSCs promoted by Hyaluronic acid was significantly increased via the TGF-β/Smad signaling pathways [54]. Also, the role of human amniotic fluid stem cells (hAFSCs) in bone tissue

repair was evaluated in 2019 by Basile et al. [55], while their exosomes was examined on a mono iodoacetate-induced animal model of osteoarthritis. For the first time, in 2019, by intra-articular injection of hAFSC exosomes in a rat model, it was confirmed that exosomes derived from hAFSC accelerate cartilage repair by promoting the migration, proliferation, and cartilage matrix synthesis [56]. Moreover, the evaluation of potential of human amniotic fluid-derived stem cells in combination with the 3D scaffold (Nano-hydroxyapatite chitosan), to treat tibial bone defects showed high proliferative and osteogenic potentials in animal model [57]. For more than 50 years, bioactive and biodegradable materials have been applied under orthopedic clinical condition [58], the assessment of biological behavior of some of these materials compared with AM, in a comparative study conducted in 2018 revealed that AM graft have significant bone regeneration induction in comparison with collagen membrane graft for tibial bone defect in large animal studies [59].

In general, by briefly looking at recent years, the most important clinical trials that used AM for orthopedic condition utilized decellular AM as an injectable material to improve healing or manage pain, can be found. Given previous clinical trials that examined the efficacy of micronized dehydrated human amniotic/chorionic membrane injection on treating chronic plantar fasciitis [60–62], Hanselman et al.' study in 2015 can be named that was conducted to compare cryopreserved hAM with corticosteroid effect to treat plantar fasciitis in 23 patients. Accordingly, 14 patients were randomized to receive the corticosteroid injection and nine patients to receive the hAM injection [63]. Liu et al., in 2018 have also evaluated the efficacy of using decellularized amniotic on preventing tendon adhesions. In this regard, a single-layer Amniotic membrane was used to reconstruct the tendon sheath defect and the flexor digitorum tendon in zone II in Henry chickens. Finally, the results of this study have indicated that amniotic membrane can effectively inhibit exogenous healing and also promote endogenous healing to prevent tendon adhesion. Moreover, although the results showed that hAM could be promising for managing plantar fasciitis pain [64], the majority of outcome's measurements showed no statistical difference between these two groups. In 2016, vines et al. in their study used cryopreserved amniotic suspension for Knee osteoarthritis (OA) in 6 patients. The results of this pilot study have indicated that a single intra-articular injection of Human amniotic suspension allografts is feasible in patients with knee OA [65]. Pain management was also performed by AM through the downregulation of pro-inflammatory cytokines such as TNF-α and IL-6. Furthermore, it was found that the activated neutrophils, and M1 & M2 macrophages could relieve pain [66]. In this regard, Castellanos et al., in 2019 used amniotic membrane/umbilical cord (AMUC) to manage pain in 20 patients with various severities of knee osteoarthritis. Subsequently, they injected 50 mg of AMUC particulate reconstituted in 2 mL of preservative-free saline into their articular space. Afterward, these patients showed a significant decrease in pain, and their physical functions have meaningfully improved [67]. Intra-articular injection of AMUC in 9 patients with facet joint syndrome have also indicated that it can be a safe and effective alternative to relieve the facet joint syndrome pain [66]. Clinical and preclinical [68] studies also showed that intra-articular injection of amniotic suspension allograft could be presented as an effective treatment for symptomatic knee OA [69]. Companies like Applied biologics® developed some products such as Flograft™ (liquid) and XWRAP™ (sheet) to minimize post-operative adhesions, reduce inflammation, and further promote soft tissue healing to protect tendons and nerves at the surgical site [70].

3.3. Ophthalmology & ocular disease

AM, as a rich source of active factors, is used in many different indications of ophthalmology such as a graft to be a scaffold for epithelial cells for surface reconstruction, in conjunctiva, and also in cornea. AM is also applied under some clinical conditions such as transplantable patch (; like contact lens) on surface as a biological cover, so this cover should be removed in specific time point, and finally, it can be used as small pieces to put in depth of corneal ulcer or crater. In addition, it has been proven that AM in all shapes of usage has the abilities to facilitate the migration, increase proliferation of epithelial cells, construct stromal tissue scarring, and decrease ocular surface inflammation [71]. in this regard, long-term safety and efficacy of AM transplant in 22 patients with bullous keratopathy were reported by Ji-Yee Siu et al. [72].

Malla et al. (2018) in their comparative study have also described three different methods as follows: primary and recurrent pterygium surgery using the combined conjunctival autograft and amniotic membrane transplantation (CAT with AMT), conjunctival autograft transplantation (CAT) alone, and amniotic membrane transplantation (AMT) alone in 142 patients. They have concluded that combination therapy using CAT and AMT meaningfully have lower rates of recurrence and postoperative complications [73]. AM was also applied as subordinate therapy as well as surgery and/or medicine, which were more reported in recent clinical studies compared to the use of AM alone. Correspondingly, Yadava et al. conducted a randomized prospective interventional study to assess the role of AMT, as an additional modulator, in primary Mitomycin C augmented trabeculectomy in 39 adult patients with uncontrolled primary glaucoma. Finally, as expected, the results indicated that amnion could provide a scaffold for the repair of acutely injured conjunctiva. Furthermore, to prevent the conjunctiva from being thin and leaky [74], Röck et al. conducted a great retrospective study in 2019 on 521 patients to compare the effectiveness of three surgical methods for primary pterygium excision including conjunctival autograft, primary closure, and AM transplantation for primary pterygium excision. Finally, their results indicated that pterygium surgery with conjunctival autograft was the most effective method with a significantly lower recurrence rate after surgery using a conjunctival autograft [75]. 16 patients with recurrent high myopic macular hole were included in this study, and they were subjected to pars plana vitrectomy with internal limiting membrane peeling and gas endotamponade before transplantation with human amniotic membrane (hAM) plug. Afterward, transplantation showed encouraging best-corrected visual acuity recovery in all the cases [76]. Over the past decade, AM as a natural derived biomaterials with ideal biological and mechanical properties was used in different research fields. In ophthalmology, as we mentioned earlier, AM can be applied and act as cover, but recently researchers tried to culture cells on it like scaffold to develop 3D culture model, to boost its healing potential. Interestingly, Che et al., in 2019 investigated a new method in constructing tissue-engineered corneal stromal in combination with keratocytes and multilayer ultrathin amniotic membrane. They have established a novel 3D biomimetic corneal model to renew the corneal stromal tissue *in vitro* model [77]. The results of these kinds of efforts are not only limited to research, but also translated to clinic. Accordingly, Banderia et al., in 2019 conducted the first clinical study to evaluate the efficiency of using cultivated conjunctival epithelium transplantation on denuded human amniotic membrane prepared by ice-cold urea, as a basement membrane scaffold for cell-based tissue-engineered treatments of ocular surface disorders. To do this, they performed this technique on two patients, and their results indicated that this method could facilitate and mainstream a minimally invasive cell-based treatment for the reconstruction of extensive ocular surface wounds [78]. From 2000 till now, Different researchers have tried to optimize and standardize some methods for AM preservation without affecting histological structure and biological properties. To date, the most employed shape of AM used in ocular surgery is dried AM, and acellular lyophilized AM is more effective in comparison with conventional intact cryopreserved AM [79]. More recently, in a clinical study conducted by Yeu et al., the safety and efficacy of cryopreserved amniotic cytokine extract (ACE) were assessed to treat 54 patients with dry eye disease. As a result they showed ACE effectiveness with highly satisfactory improvements in the clinical signs and symptoms [80].

Besides, some reports have suggested that amnion-derived cells also are the effective sources of potent cells with the potential of being used in regenerative medicine [81]. Higa et al., in 2019 evaluated the effects of human amniotic membrane–derived fibroblast on corneal epithelium regeneration, and showed the promoted healing effect on corneal wound [82]. The potential of human amniotic epithelial cells (hAECs) transplant in autoimmune uveitis was investigated by Li group, and their results indicated that subretinal hAEC transplantation is a new potentially therapeutic strategy for autoimmune uveitis (AU) as well as the related ocular inflammatory diseases [83]. Another possible source of potent cells was isolated from AF with ability of differentiation and clinical application, with no risk of tumorigenesis becoming a promising therapeutic option for human tissue repair. Recently, AF in combination with artificial tears or soft contact lens were applied to decrease severe dry eye disease symptoms in 11 patients [84]. Due to the long journey with using, assessing, and clinical application of AM in ocular related diseases, different companies have tried to industrialize acellular AM for ocular clinical conditions. For instance; Prokera (or Prokera Slim; TissueTech Inc, Doral, FL), which was approved by food and drug administration (FDA) as suture less cryopreserved membrane clipped to a polycarbonate ring introduced to market, for the purpose of curing ocular conditions such as keratitis, moderate to severe dry eye disease, recurrent corneal erosions, filamentary keratitis, persistent epithelial defects, neurotrophic corneas, herpetic ulcers, and many other ocular surface diseases such as chemical burns and Stevens-Johnson syndrome. However, its effects were lower than expected, which were also reported in the findings of a study by Shafer et al., in 2019 conducted to evaluate the result of transplantation Prokera in 6 patients with Sjögren syndrome (SS). Moreover, the results of slit-lamp examination of the cornea and conjunctiva, dry eye symptoms, and visual acuity showed that cryopreserved AM could have a temporary beneficial effect on SS patients with ocular surface disease [85]. Ambio2® from Katena Inc, is another example which is dehydrated AM was designed for ocular surface disease and pterygium. In this regard, Miyakoshi et al. (2019) conducted a prospective controlled animal study to asses this product with hyper-dry amniotic membrane for comparing the histological changes after its subconjunctival implantation. Finally, their results revealed that both groups were entirely absorbed with no symptom of scarring within a six-month follow up period, and the histological results were almost equivalent in both groups [86]. Despite various reports on using AM as an adjuvant therapy in eye surgeries and treatments, no considerable effects have been reported in some studies. Accordingly, Eslani et al. performed a randomized clinical trial in 2018 to use AM as well as chloramphenicol, betamethasone, homatropine, oral vitamin C, and doxycycline (standard care) to increase epithelial healing and the final visual acuity in 60 patients with Severe Ocular Chemical Injuries, their results showed that there was no difference between this group in comparison with the control group that received the standard care [87]. Generally, the available data indicated the results of effectiveness, strongly were related to the kinds of ocular diseases.

3.4. Urology

Based on the number of surveys conducted or on going in clinical studies, urology also have a big part in studies performed in the field of using AM. The first report on glutaraldehyde-stabilized AM was done for regeneration bladder in 1982 [88]. Recently, power of cells isolated from AM or cells cultivated on human AM were explored for urological and neurological diseases, as well. Accordingly, Gottipamula et al., in 2019 observed the anti-fibrotic character of human amniotic epithelial cells (hAECs) on urethral stricture fibroblast cells [89]. Chen et al. have seeded allogeneic bone marrow mesenchymal cells (BMSCs) and/or endothelial progenitor cells (EPCs) on decellularized human amniotic scaffolds (dHAS) as a treatment for the urethral defect in dogs. Subsequently, they concluded that dHAS seeded with allogeneic BMSCs + EPCs or EPCs can more effectively repair a 3-cm circumferential urethral defect in big animal model [90]. More recently, stem cells derived from amniotic fluid were investigated for their differentiation potentials into renal proximal tubular epithelial-like cells [91]. Therapeutic potentials of these cells were evaluated for renal regeneration by Yu group in 2019, along with regeneration of glomerular/renal tubules and low expression levels of pro-inflammatory cytokines and fibrotic factors in a partially resected kidney *in vivo* [92]. Moreover, George et al., in 2019 demonstrated a structural improvement after AFSC transplantation to recover renal function with chronic kidney disease in a rat model [93]. The isolated human amnion-derived MSC can also ameliorate renal damage, reduce fibrosis and apoptosis, and increase proliferation in kidney failure in an In Vivo study [94]. An enormous clinical study was performed by Razdan et al., in 2019 to assess efficacy of dehydrated hAM around nerve bundles (NVB) during robotic-assisted laparoscopic radical prostatectomy (RALP). In this study, 1400 patients were selected and 700 patients were subjected to dHAM allograft wrapped around the NVB transplantation. The results of this enormous clinical study revealed that the use of dHAM had an overall higher satisfactory potency outcomes by passing one year from RALP [95]. Another clinical study was developed in 2019 by Barski et al. to evaluate the efficacy and safety of dehydrated hAM placed around the neurovascular bundle and vesicoureteral anastomosis (VUA) during RP for the treatment of the localized prostate cancer. Their outcomes showed that dHAM could be considered as a suitable scaffold for faster improving VUA healing and following recovery [96].

3.5. Oral and periodontal field

The chief goal of using AM in dentistry is to accelerate tissue regeneration process. As we mentioned earlier, and also as shown by other researchers, properties of AM such as immunomodulation, coverage with the antimicrobial effect, and enriched with different GF and proteins have indicated that AM could led to the increased gingival tissue regeneration, the facilitated migration, and the reinforced adhesion [97], as a proper root coverage [98–100]. In addition, reconstruction of oral cavity (tongue, buccal mucosa, vestibule, palatal mucosa, and floor of the mouth) can be achieved by the dehydrated AM transplantation [101]. Also, a significant reduction was reported by researchers in the need for second surgery upon root coverage, which showed Amnion-Chorion membrane on the root surface in comparison with using PRF (platelet rich fibrin), as a bio-material alternative [102]. Comparison of healing potential of this common GF source (PRF) used by dentists in laboratory preparation set-up, was also evaluated in combination with AM by Kaur et al., in 2018. Correspondingly, this study showed a significant improvement for grade 2 furcation defect using PRF and AM in combination compared to PRF alone [103]. Interestingly, a clinical study performed in 2018 on 10 patients with periodontal Grade II buccal furcation defects showed the efficacy of demineralized freeze dried bone allograft (DFDBA) and bovine derived xenogenic bone graft (BDX) with amniotic membrane (AM). Their results have shown a significant pocket depth reduction, bone fill, and percentage of gain using both treatments [104]. It seems that dehydrated and cryopreserved AM are used more than fresh, gel or other physical shapes in the field of dentistry. The accumulated data showed that AM, as an adjuvant therapy, could be effective on oral defect, which can boost and help the bone graft [105] and also accelerate wound healing after dental implant surgery.

3.6. Oncology & cancer field

Different properties of the AM such as pro-apoptotic, anti-angiogenesis, cell-cycle arrest, and immune-regulatory characteristics have made it a suitable candidate for cancer-related investigations. As we mentioned above, oncology-hematology has few share of AM-related studies but seems to have more potential challenge which should be discovered with more research. Here, we try to show the current

situation of studies and more challengeable part of this area.

Seo et al., in 2008 for the first time conducted a study on the potential of anti-carcinogenic properties of AM [106]. Accumulated studies have found the anti-cancer mechanism for AM through the secretion of different proteins and cytokines. Collagen VIII, Thrombospondin (TSP), Tissue Inhibitors of Metalloproteinases (TIMPs) (especially TIMP-2), Plasminogen activator inhibitors PAI-1 and PAI-2, Interleukin-10 (IL-10), and Interleukin-1 receptor antagonist (IL1RN) can also mediate the role of anti-angiogenesis. Human MSC isolated from AM, excrete various anti-proliferative factors and cytokines such as GM-CSF (granulocyte macrophage-colony stimulating factor), IL-6 (interleukin-6), IFN-α, and –β (interferon-α and –β). In addition, pro-apoptotic properties suggested to be performed through the inhibition of HSP90 and activation of Bcl-2/caspase pathway [107]. Different assessments were designed by different groups to show some anti-tumoral properties such as *in vitro* co culture evaluation with the cells (like MSC and/or amniotic epithelial cells) isolated from AM or with the growth factors expressed by AM [108], and hAM-conditioned medium was also used as a source of different bioactive molecules to inhibit proliferation [109]. Whole membrane was investigated in different animal models in the fresh, dehydrated or cryopreserved ones [110]. For example; the risk of tumor recurrence after partial tumor resection was examined by Alvim et al. after the treatment with dehydrated commercial human amnion/chorion membrane (dHACM) allografts on prostate and bladder cancer in both *in vitro* and *in vivo* models. Besides, they evaluated tumor growth using different immunohistochemistry studies (IHC) between the group that received membrane and the group that was not subjected to implant. Afterward, the result indicated no significant differences in the volume and weight of tumors between these two groups and also no change was observed between the expressions of CD31 as a marker for angiogenesis and Ki67 as a marker of proliferation in in-vivo model. It should be noted that, their results showed a lower level of the expression of Vimentin in prostate cancer cell lines after dHACM treatment *in vitro* as well as a faster tissue remodeling and microenvironment re-composition after AM transplantation in animal model [111]. A comparative study performed by Modaresifar et al., in 2017 showed that the lower viability of hAECs was after cryopreservation to about 50%, but the anti-proliferative effect of the cryopreserved AM was similar to a fresh tissue [110]. The potential of using this cells (hAECs) was evaluated in different cancer models like being used on epithelial ovarian cancer cells in both *in vivo* and *in vitro* models by BU et al. In addition, the results proved that hAECs have a potential application mediated by hAEC-secreted TGF-β1-induced cell cycle arrest [112]. More recently, in different studies, amniotic fluid-derived mesenchymal stem cells, as a novel cancer therapy, was evaluated in cervical cancer [113], prostate cancer [114], and ovarian cancer [115]. Exosomes derived amnion epithelial cells (AEC) isolated from the conditioned medium was enriched by embryonic stem cell markers, and different analyses showed a wide variety of proteins involved in immunomodulatory responses [116]. Exosomes derived AEC, placental, umblical cord wharton's jelly, and umblical cord MSC in cancer therapy were all reviewed recently by Vakhshiteh et al. [117]. They reported in their *in vitro* set-up, in ovary and breast cancer cells treated with human umblical cord MSC- derived, which indicated cancer cells growth promotion by transferring MMP-2 enzyme, the placental exosomes derived MSC showed the increased migration and angiogenesis in endothelial cells. On the contrary, they have also reported in both *in vitro* and *in vivo* studies using umblical cord wharton's jelly MSC-derived extracellular vesicle in bladder cancer, which demonstrated an inhibition in tumor growth by down-regulation of AKt protein kinase as well as the up-regulation of caspase-3. Therefore, in conclusion, it seems that the use of the AM derived exosomes in the field of cancer therapy needs more studies to elucidate the exact mechanism of action as well as the way that we can use them to inhibit cancer cells progression.

4. Survey of clinical trials

As we discussed in the last section, numerous studies have been designed to test AM alone or in combination with different types of cells in preclinical and clinical trials, in order to show the healing potential under various clinical conditions. Results of a literature survey on clinical trials from 2015 to 2020 (with special focus on the two last years) using Amniotic membrane, Amniotic dressing, Amniotic membrane transplantation, and Amniotoc membrane graft separate with regenerative medicine, stem cells, tissue engineering, cell therapy as keywords in clinicaltrial.gov and NCBI clinical trial section, are shown in Table 1.

Notably, they have been sorted on the basis of condition, clinical phase, year, number of claimed enrollment, country, and disease types. The results revealed that a total of 10291 patients were enrolled or considered to be a part for 87 clinical trial studies performed from 2015 to 2020. One of the most important clinical trials, namely Safety and Clinical outcomes with amniotic and umbilical cord tissue therapy for numerous medical conditions, was recently started in USA and then supported by R3 stem cell clinic and enrolling 5000 patients. afterward, this study was approved by Alion accredited Institutional Review Board, Solutions IRB (institutional review board, also known as an independent ethics committee) to use growth factors, exosomes, cytokines, and stem cells derived from umblical cord tissue and amniotic membrane for orthopedic condition (injection), neurologic disease (IV infusion and intranasal procedure), urologic (injection), autoimmune (IV infusion), cardiac (IV infusion), pulmonary (IV Infusion plus Nebulizer), and renal (IV infusion) clinical condition. In this regard, the patients were selected non-randomly, followed up for 1, 3, 6, 9, 12, 18, 24, 30, and 36 months, and then on average each year, up to 10 years after the intervention. It seems that this is the most extensive clinical set up of application AM to date, which was prepared in the lab approved by food and drug administration. The second large study was the clinical trial with dried amniotic membrane for post-prostatectomy incontinence that was designed to undertake 328 patients. In this study, dAM was used around the neurovascular bundle (NVB) and vesicourethral anastomosis (VUA) during radical retropubic prostatectomy (RRP) in a tertiary centre in Germany. Both above-mentioned clinical studies were started in 2019 and the status is in the phase of not recruiting patients, yet. In total, 16 trials reported in clinical trial.gov and 14 published reports based on NCBI clinical trial were completed during the last 5 years, which have 1275 actual enrollments treated by AM. Approximately 50% of these completed trial were performed in wound area. It should be noted that 14% of clinical trials were supported by companies. In this regard, CLARIX FLO, BioXclude™, ViaShield™ amnion patch, ReNu™ allograft, Skye Barrier, AmnioExCel dressing, ReNu Injection, AMNIOEXCEL®, NuShield and NuCel® allograft were the products considered in the clinical studies to be assessed for their efficacy on wound healing, ophthalmology, orthopedic, and even neurological diseases. The Blue Cross Blue Shield Association[1] published a report in terms of the 91 available articles and guidelines in the March of 2020 that reviewed the recent policy of using hAM under different clinical conditions. Human AM graft can be considered for the treatment of lower-extremity diabetic skin ulcers, certain (not all) ophthalmic indications, and some of orthopedic conditions (including tendonitis, plantar fasciitis, cartilage damage, and for the alleviation of pain and stiffness in patients with osteoarthritis), if the medical appropriateness criteria are met. The injection of micronized or particulated human AM fluid and/or amniotic fluid for the treatment of osteoarthritis and plantar fasciitis is considered in investigations. The list of the approved and under-development commercial products derived AM is shown in Table 2. The possibility

[1] The Blue Cross Blue Shield Association is a federation of 36 separate United States health insurance companies that provide health insurance in the United States to more than 106 million people.

of long-term preservation at room temperature after drying process is the main reason for the production of numerous dried-based products. 31 out of 51 available and under development products derived AM were considered to be used for acute and chronic wounds. Reviewing these products showed that the current challenge is producing a cost-effective procedure to produce cheaper products with more efficiency.

5. Conclusion & future prospect

Amniotic membranes have many promising applications in regenerative medicine due to having the potential of natural EMC that contains active molecules. Given various clinical data supporting the benefits of amnion in wound healing, ophthalmology, orthopedic, urology, and periodontal, clinician as well as researchers should more emphasize translation to market. Since 2000, FDA defined a regulatory framework for Human Cells and Tissue Products, or HCT/P's, amniotic membrane products (such as dehydrated and decellularized AM for wound dressing and ocular repair) qualified under "361 exemption", which means that as long as they are safe, they can be marketed without undergoing strict testing to prove their efficacy [118]. If the products could not be qualified for the 361 exemption (such as micronized cryopreserved AM), regulation might be continued with 351 pathways as a drug, device, and/or a biological product, which require a severe premarket approval [119]. Overview of global market reports in this field showed that cryopreserved amniotic membrane had a significant market share in the last year. Accordingly, this data was confirmed by finding of a review on recent clinical studies. This might be resulted from some issues related to preservations and contamination in other forms. As expected, global market analysis reported a wide application of amniotic membrane transplantation in ophthalmic surgeries, a high demand for amniotic membrane in wound repair and reconstruction, and a rising demand for biocompatible scaffolds [120]. It should be noted that recent clinical studies showed a more efficacy and a lower cost for the use of some alternatives for wound healing such as skin fish in comparison with AM. So, it seems that identifying the new correct indication, cost-effectiveness, and clinical efficacy is the major challenge through further developments. We believe that information reviewed in this study are valuable to obtain the picture of the latest clinical trends and are also helpful for clinicians and related manufacturing companies.

Author disclosure

All authors were involved in the research and the authors declare no conflict of interest.

Financial support and sponsorship

Nil.

Acknowledgement

We apologize to all researchers whose work could not be cited due to space limitation.

Abbreviations and acronyms

AM:	Amniotic membrane;
RM	Regenerative Medicine;
AF	Amniotic fluid
AEC	Amniotic epithelial cell
AMSC	Amniotic mesenchymal stem cells
AMT	Amniotic membrane transplantation
CAT	conjunctival autograft transplantation
hAM	human amniotic membrane
ACE	amniotic cytokine extract
PG	Platelet gel
PPP	Platelet poor plasma
CBPG	Cord blood derived platelet gel
PRP	platelet poor plasma
hAECs	human amniotic epithelial cells
SS	Sjögren syndrome
TSP	Thrombospondin
TIMPs	Tissue Inhibitors of Metalloproteinases
PAI	Plasminogen activator inhibitors
IL:	Interleukin
ILRN	Interleukin receptor antagonist
GM-CSF	granulocyte macrophage-colony stimulating factor
IFN	interferon
HSPs	heat shock proteins
dHACM	human amnion/chorion membrane
dHAS	decellularized human amniotic scaffolds
BMSCs	bone marrow mesenchymal cells
EPCs	endothelial progenitor cells
NVB	nerve bundles
VUA	vesicoureteral anastomosis
hAMSCs	human amnion membrane mesenchymal stem cells
hAFSCs	human amniotic fluid stem cells
OA	osteoarthritis
AMUC	amniotic membrane/umbilical cord
PRF	platelet rich fibrin
DFDBA	demineralized freeze dried bone allograft
BDX	bovine derived xenogenic bone graft

References

[1] M. Abbasi-Kangevari, et al., Potential therapeutic features of human amniotic membrane mesenchymal stem cells in multiple sclerosis: immunomodulation, inflammation suppression, angiogenesis promotion, oxidative stress inhibition, neurogenesis induction, MMPs regulation, and remyelination stimulation, Front. Immunol. 10 (2019) 238.
[2] A.C. Mamede, et al., Amniotic membrane: from structure and functions to clinical applications, Cell Tissue Res. 349 (2) (2012) 447–458.
[3] Y. Nanbu, et al., CA 125 in the epithelium closely related to the embryonic ectoderm: the periderm and the amnion, Am. J. Obstet. Gynecol. 161 (2) (1989) 462–467.
[4] S. Díaz-Prado, et al., Isolation and characterization of mesenchymal stem cells from human amniotic membrane, Tissue Eng. C Methods 17 (1) (2011) 49–59.
[5] M. Caruso, M. Evangelista, O. Parolini, Human term placental cells: phenotype, properties and new avenues in regenerative medicine, Int. J. Mole. Cell. Med. 1 (2) (2012) 64.
[6] G. Pratama, et al., Changes in culture expanded human amniotic epithelial cells: implications for potential therapeutic applications, PloS One 6 (11) (2011).
[7] L. Centurione, et al., Mapping of the human placenta: experimental evidence of amniotic epithelial cell heterogeneity, Cell Transplant. 27 (1) (2018) 12–22.
[8] J. Hori, et al., Immunological characteristics of amniotic epithelium, Cornea 25 (2006) S53–S58.
[9] B.F. King, Distribution and characterization of anionic sites in the basal lamina of developing human amniotic epithelium, Anat. Rec. 212 (1) (1985) 57–62.
[10] N.S. Corsini, A. Martin-Villalba, Integrin alpha 6: anchors away for glioma stem cells, Cell Stem Cell 6 (5) (2010) 403–404.
[11] P. Rousselle, et al., Laminin 5 binds the NC-1 domain of type VII collagen, J. Cell Biol. 138 (3) (1997) 719–728.
[12] H. Niknejad, et al., Properties of the amniotic membrane for potential use in tissue engineering, Eur. Cell. Mater. 15 (2008) 88–99.
[13] C. McDonald, C. Siatskas, C.C. Bernard, The emergence of amnion epithelial stem cells for the treatment of multiple sclerosis, Inflamm. Regen. 31 (3) (2011) 256–271.
[14] P. Li, et al., Cell therapeutic potential of human amniotic epithelial cells. AIP Conference Proceedings., AIP Publishing LLC, 2019.
[15] G. Leila, et al., Ensaio clínico randomizado sobre o efeito do enxerto de membrana amniótica sobre o processo de cicatrização após fistulotomia anal, J. Coloproctol. (Rio de Janeiro) 37 (3) (2017) 187–192.
[16] W. Tettelbach, et al., A confirmatory study on the efficacy of dehydrated human amnion/chorion membrane dHACM allograft in the management of diabetic foot ulcers: a prospective, multicentre, randomised, controlled study of 110 patients from 14 wound clinics, Int. Wound J. 16 (1) (2019) 19–29.
[17] S. Ghalei, et al., Enhanced cellular response elicited by addition of amniotic fluid to alginate hydrogel-electrospun silk fibroin fibers for potential wound dressing application, Colloids Surf. B Biointerfaces 172 (2018) 82–89.
[18] M. Gholipourmalekabadi, et al., Silk fibroin/amniotic membrane 3D bi-layered artificial skin, Biomed. Mater. 13 (3) (2018), 035003.

[19] M. Momeni, et al., In vitro and in vivo investigation of a novel amniotic-based chitosan dressing for wound healing, Wound Repair Regen. 26 (1) (2018) 87–101.
[20] M.S. Rahman, et al., Characterization of burn wound healing gel prepared from human amniotic membrane and Aloe vera extract, BMC Compl. Alternative Med. 19 (1) (2019) 115.
[21] Z. Kakabadze, et al., Bone marrow stem cell and decellularized human amniotic membrane for the treatment of nonhealing wound after radiation therapy, Exp Clin Transplant 17 (2019) 92–98.
[22] D. Kitala, et al., Amniotic cells share clusters of differentiation of fibroblasts and keratinocytes, influencing their ability to proliferate and aid in wound healing while impairing their angiogenesis capability, Eur. J. Pharmacol. 854 (2019) 167–178.
[23] S. John, et al., Development of a tissue-engineered skin substitute on a base of human amniotic membrane, J. Tissue Eng. 10 (2019), 2041731418825378.
[24] M. Fukutake, et al., Human amniotic fluid stem cells have a unique potential to accelerate cutaneous wound healing with reduced fibrotic scarring like a fetus, Hum. Cell 32 (1) (2019) 51–63.
[25] H. Zhou, et al., Feasibility of repairing full-thickness skin defects by iPSC-derived epithelial stem cells seeded on a human acellular amniotic membrane, Stem Cell Res. Ther. 10 (1) (2019) 155.
[26] S. Farzamfar, et al., Promotion of excisional wound repair by a menstrual blood-derived stem cell-seeded decellularized human amniotic membrane, Biomed. Eng. Lett. 8 (4) (2018) 393–398.
[27] Y. Sun, et al., Urine-derived stem cells secreting small extracellular vesicles loaded in acellular amniotic membrane for wound healing promotion in aged mice, 2019. Available at: SSRN 3478068.
[28] B. Zhao, et al., Exosomal MicroRNAs derived from human amniotic epithelial cells accelerate wound healing by promoting the proliferation and migration of fibroblasts, Stem Cell. Int. 2018 (2018).
[29] S. Xiao, et al., IL-10 gene-modified human amniotic mesenchymal stem cells augment regenerative wound healing by multiple synergistic effects, Stem Cell. Int. 2019 (2019).
[30] S.V. Murphy, et al., Solubilized amnion membrane hyaluronic acid hydrogel accelerates full-thickness wound healing, Stem Cells Translat. Med. 6 (11) (2017) 2020–2032.
[31] J. Kshersagar, et al., Decellularized amnion scaffold with activated PRP: a new paradigm dressing material for burn wound healing, Cell Tissue Bank. 19 (3) (2018) 423–436.
[32] S.V. Murphy, et al., Amnion membrane hydrogel and amnion membrane powder accelerate wound healing in a full thickness porcine skin wound model. Stem Cells Translational Medicine, 2019.
[33] C.R.S. Prakoeswa, et al., The efficacy of topical human amniotic membrane-mesenchymal stem cell-conditioned medium (hAMMSC-CM) and a mixture of topical hAMMSC-CM + vitamin C and hAMMSC-CM + vitamin E on chronic plantar ulcers in leprosy:a randomized control trial, J. Dermatol. Treat. 29 (8) (2018) 835–840.
[34] S. Dhall, T. Hoffman, M. Sathyamoorthy, et al., A viable lyopreserved amniotic membrane modulates diabetic wound microenvironment and accelerates wound closure, Adv. Wound Care 8 (8) (2019) 355–367, https://doi.org/10.1089/wound.2018.0931.
[35] T.B. Bemenderfer, et al., Effects of cryopreserved amniotic membrane-umbilical cord allograft on total ankle arthroplasty wound healing, J. Foot Ankle Surg. 58 (1) (2019) 97–102.
[36] S. Magnusson, et al., Regenerative and antibacterial properties of acellular fish skin grafts and human amnion/chorion membrane: implications for tissue preservation in combat casualty care, Mil. Med. 182 (suppl_1) (2017) 383–388.
[37] R.S. Kirsner, et al., Fish skin grafts compared to human amnion/chorion membrane allografts: a double-blind, prospective, randomized clinical trial of acute wound healing, Wound Repair Regen. 28 (1) (2020) 75–80.
[38] N.K. Radwan, N.F. Ibrahim, A.S. Bashandy, Uses of gamma irradiated amniotic membrane as an alternative method in psoriasis treatment, Cell Tissue Bank. 19 (4) (2018) 733–741.
[39] A. Piccin, et al., Platelet gel: a new therapeutic tool with great potential, Blood Transfus 15 (4) (2017) 333–340.
[40] Y. Mu, X. Wu, Z. Hao, Comparative evaluation of mesenchymal stromal cells from umbilical cord and amniotic membrane in xeno-free conditions, BMC Cell Biol. 19 (1) (2018) 27.
[41] V. Parazzi, L. Lazzari, P. Rebulla, Platelet gel from cord blood: a novel tool for tissue engineering, Platelets 21 (7) (2010) 549–554.
[42] A. Piccin, et al., Impressive tissue regeneration of severe oral mucositis post stem cell transplantation using cord blood platelet gel, Transfusion 57 (9) (2017) 2220–2224.
[43] J.L. Alio, A.E. Rodriguez, D. WrobelDudzinska, Eye platelet-rich plasma in the treatment of ocular surface disorders, Curr. Opin. Ophthalmol. 26 (4) (2015) 325–332.
[44] H.C. You J, M. Hoque, C. Petsoglou, G. Sutton, Human platelets and derived products in treating ocular surface diseases - a systematic review, Clin. Ophthalmol. 14 (2020) 3195–3210.
[45] M. Wang, et al., Amniotic fluid-derived stem cells mixed with platelet rich plasma for restoration of rat alveolar bone defect, Acta Biochim. Biophys. Sin. 49 (3) (2017) 197–207.
[46] L. Yang, et al., Platelet poor plasma gel combined with amnion improves the therapeutic effects of human umbilical cordderived mesenchymal stem cells on wound healing in rats, Mol. Med. Rep. 16 (3) (2017) 3494–3502.
[47] A. Piccin, et al., Healing of a soft tissue wound of the neck and jaw osteoradionecrosis using platelet gel, Regen. Med. 11 (5) (2016) 459–463.
[48] A. Piccin, et al., Severe skin radiodermatitis fully healed with the use of platelet gel and a hyperbaric chamber, Blood Transfus 14 (6) (2016) 552–554.
[49] D.W. Taylor, et al., A systematic review of the use of platelet-rich plasma in sports medicine as a new treatment for tendon and ligament injuries, Clin. J. Sport Med. 21 (4) (2011) 344–352.
[50] A. Papait, et al., Mesenchymal stromal cells from fetal and maternal placenta possess key similarities and differences: potential implications for their applications in regenerative medicine, Cells 9 (1) (2020).
[51] K.W. Marvin, et al., Expression of angiogenic and neurotrophic factors in the human amnion and choriodecidua, Am. J. Obstet. Gynecol. 187 (3) (2002) 728–734.
[52] N. Hendrijantini, P. Hartono, Phenotype characteristics and osteogenic differentiation potential of human mesenchymal stem cells derived from amnion membrane (HAMSCs) and umbilical cord (HUC-MSCs), Acta Inf. Med. 27 (2) (2019) 72.
[53] T. Gualdi, et al., In vitro osteodifferentiation of intact human amniotic membrane is not beneficial in the context of bone repair, Cell Tissue Bank. (2019) 1–12.
[54] L.-T. Zhang, et al., Hyaluronic acid promotes osteogenic differentiation of human amniotic mesenchymal stem cells via the TGF-β/Smad signalling pathway, Life Sci. 232 (2019) 116669.
[55] M. Basile, F. Marchegiani, S. Novak, I. Kalajzic, R. Di Pietro, et al., Human amniotic fluid stem cells attract osteoprogenitor cells in bone healing, J. Cell. Physiol. 235 (5) (2019) 4643–4654, https://doi.org/10.1002/jcp.29342.
[56] M. Zavatti, et al., Comparison of the Therapeutic Effect of Amniotic Fluid Stem Cells and Their Exosomes on Monoiodoacetate-induced Animal Model of Osteoarthritis, BioFactors, 2019.
[57] E.E. Mohammed, et al., Combination of human amniotic fluid derived-mesenchymal stem cells and nano-hydroxyapatite scaffold enhances bone regeneration, Open Access Macedonian J. Med. Sci. 7 (17) (2019) 2739.
[58] M. Navarro, a Michiardi, O. Castaño, J.A. Planell, Biomaterials in orthopaedics, J. R. Soc. Interface 5 (27) (2008) 1137–1158.
[59] S. Koushaei, et al., Histological comparison of new bone formation using amnion membrane graft versus resorbable collagen membrane: an animal study, J. Oral Implantol. 44 (5) (2018) 335–340.
[60] C.M. Zelen, A. Poka, J. Andrews, Prospective, randomized, blinded, comparative study of injectable micronized dehydrated amniotic/chorionic membrane allograft for plantar fasciitis—a feasibility study, Foot Ankle Int. 34 (10) (2013) 1332–1339.
[61] K.J. Alden, S. Harris, B. Hubbs, K. Kot, N.B. Istwan, D. Mason, et al., Micronized dehydrated human amnion chorion membrane injection in the treatment of knee osteoarthritis—a large retrospective case series, J. Knee Surg. (1938–2480 (Electronic) 1538–8506 (Linking)) (2019).
[62] T.D. Luo, et al., Evaluation of percutaneous intradiscal amniotic suspension allograft in a rabbit model of intervertebral disc degeneration, Spine 44 (6) (2019) E329–E337.
[63] C. Liu, et al., Experimental study of tendon sheath repair via decellularized amnion to prevent tendon adhesion, PloS One 13 (10) (2018), e0205811.
[64] A.E. Hanselman, J.E. Tidwell, R.D. Santrock, Cryopreserved human amniotic membrane injection for plantar fasciitis: a randomized, controlled, double-blind pilot study, Foot Ankle Int. 36 (2) (2015) 151–158.
[65] J.B. Vines, et al., Cryopreserved amniotic suspension for the treatment of knee osteoarthritis, J. Knee Surg. 29 (2016) 443–450, 06.
[66] D.S. Bennett, Cryopreserved amniotic membrane and umbilical cord particulate for managing pain caused by facet joint syndrome: a case series, Medicine 98 (10) (2019).
[67] R. Castellanos, S. Tighe, Injectable Amniotic membrane/umbilical cord particulate for knee osteoarthritis: a prospective, single-center pilot study, Pain Med. 20 (11) (2019) 2283–2291.
[68] I. Marino-Martínez, et al., Human amniotic membrane intra-articular injection prevents cartilage damage in an osteoarthritis model, Exp. Therapeutic Med. 17 (1) (2019) 11–16.
[69] J. Farr, et al., A randomized controlled single-blind study demonstrating superiority of amniotic suspension allograft injection over hyaluronic acid and saline control for modification of knee osteoarthritis symptoms, J. Knee Surg. 32 (11) (2019) 1143–1154.
[70] https://www.appliedbiologics.com/.
[71] I. Rahman, et al., Amniotic membrane in ophthalmology: indications and limitations, Eye 23 (10) (2009) 1954–1961.
[72] G.D.J.-Y. Siu, K.W. Kam, A.L. Young, Amniotic membrane transplant for bullous keratopathy: confocal microscopy & anterior segment optical coherence tomography. Seminars in Ophthalmology, Taylor & Francis, 2019.
[73] T. Malla, J. Jiang, K. Hu, Clinical outcome of combined conjunctival autograft transplantation and amniotic membrane transplantation in pterygium surgery, Int. J. Ophthalmol. 11 (3) (2018) 395.
[74] U. Yadava, et al., Simultaneous use of amniotic membrane and Mitomycin C in trabeculectomy for primary glaucoma, Indian J. Ophthalmol. 65 (11) (2017) 1151.
[75] T. Röck, et al., A retrospective study to compare the recurrence rate after treatment of pterygium by conjunctival autograft, primary closure, and amniotic membrane transplantation, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res.: Int. Med. J. Exp. Clin. Res. 25 (2019) 7976.
[76] T. Caporossi, et al., HUMAN AMNIOTIC MEMBRANE TO CLOSE RECURRENT, HIGH MYOPIC MACULAR HOLES IN PATHOLOGIC MYOPIA WITH AXIAL LENGTH OF≥ 30 Mm, Retina, Philadelphia, Pa., 2019.

[77] X. Che, et al., A novel tissue-engineered corneal stromal equivalent based on amniotic membrane and keratocytes, Invest. Ophthalmol. Visual Sci. 60 (2) (2019) 517–527.
[78] F. Bandeira, et al., Urea-De-epithelialized human amniotic membrane for ocular surface reconstruction, Stem Cells Translat. Med. 8 (7) (2019) 620–626.
[79] D. Costa, et al., A Multicenter Retrospective Study on Cryopreserved Amniotic Membrane Transplantation for the Treatment of Complicated Corneal Ulcers in the Dog, Veterinary ophthalmology, 2019.
[80] E. Yeu, et al., Safety and efficacy of amniotic cytokine extract in the treatment of dry eye disease, Clin. Ophthalmol. 13 (2019) 887.
[81] A. Umezawa, et al., Amnion-derived Cells as a Reliable Resource for Next-Generation Regenerative Medicine, Placenta, 2019.
[82] K. Higa, et al., Effects of amniotic membrane–derived fibroblast supernatant on corneal epithelium, Invest. Ophthalmol. Visual Sci. 60 (12) (2019) 3718–3726.
[83] J. Li, et al., Subretinal transplantation of human amniotic epithelial cells in the treatment of autoimmune uveitis in rats, Cell Transplant. 27 (10) (2018) 1504–1514.
[84] C. Ming, et al., A pilot study of the short term effectiveness and safety of amniotic fluid in severe dry eye disease, Med. Hypothesis, Discov. Innovation (MEHDI) Ophthalmol. 8 (2) (2019) 81.
[85] B. Shafer, et al., The use of self-retained, cryopreserved amniotic membrane for the treatment of Sjögren syndrome: a case series, Digit. J. Ophthalmol.: DJO 25 (2) (2019) 21.
[86] A. Miyakoshi, et al., Histological equivalence of a hyper-dry amniotic membrane and the Ambio2TM after implantation in the rabbit conjunctiva, Ophthalmic Res. (2019) 1–4.
[87] M. Eslani, et al., Amniotic membrane transplantation in acute severe ocular chemical injury: a randomized clinical trial, Am. J. Ophthalmol. 199 (2019) 209–215.
[88] M.A. Norris, et al., Bladder reconstruction in rabbits with glutaraldehyde-stablilized amniotic membranes, Urology 19 (6) (1982) 631–635.
[89] S. Gottipamula, S. Sundarrajan, K. Kumar Chokalingam, The effect of human amniotic epithelial cells on urethral stricture fibroblasts, J. Clinc. Trans. Res. 5 (1) (2019) 44.
[90] C. Chen, et al., Transplantation of amniotic scaffold-seeded mesenchymal stem cells and/or endothelial progenitor cells from bone marrow to efficiently repair 3-cm circumferential urethral defect in model dogs, Tissue Eng. 24 (1–2) (2017) 47–56.
[91] E. Minocha, C.P. Chaturvedi, S. Nityanand, Renogenic characterization and in vitro differentiation of rat amniotic fluid stem cells into renal proximal tubular- and juxtaglomerular-like cells, in: Vitro Cellular & Developmental Biology-Animal, 2019, pp. 138–147, 55(2).
[92] N.-h. Yu, et al., *In* vivo safety and regeneration of long-term transported amniotic fluid stem cells for renal regeneration, Tissue Eng. Regenerat. Med. 16 (1) (2019) 81–92.
[93] S.K. George, et al., Effect of human amniotic fluid stem cells on kidney function in a model of chronic kidney disease, Tissue Eng. 25 (21–22) (2019) 1493–1503.
[94] B. Cetinkaya, et al., Effects of human placental amnion derived mesenchymal stem cells on proliferation and apoptosis mechanisms in chronic kidney disease in the rat, Int. J. Stem Cell 12 (1) (2019) 151.
[95] S. Razdan, et al., A matched and controlled longitudinal cohort study of dehydrated human amniotic membrane allograft sheet used as a wraparound nerve bundles in robotic-assisted laparoscopic radical prostatectomy: a puissant adjunct for enhanced potency outcomes, J. Robotic Surg. 13 (3) (2019) 475–481.
[96] D. Barski, et al., Application of dried human amnion graft to improve post-prostatectomy incontinence and potency: a randomized exploration study protocol, Adv. Ther. 37 (1) (2020) 592–602.
[97] I. Velez, et al., Cryopreserved amniotic membrane for modulation of periodontal soft tissue healing: a pilot study, J. Periodontol. 81 (12) (2010) 1797–1804.
[98] https://clinicaltrials.gov/ct2/show/NCT03836378.
[99] https://clinicaltrials.gov/ct2/show/NCT03095976.
[100] A. Sharma, K. Yadav, Amniotic membrane - a Novel material for the root coverage: a case series, J. Indian Soc. Periodontol. 19 (4) (2015) 444–448.
[101] R. Mohan, A. Bajaj, M. Gundappa, Human amnion membrane: potential applications in oral and periodontal field, J. Int. Soc. Prev. Community Dent. 7 (1) (2017) 15–21.
[102] S.S. Shetty, A. Chatterjee, S. Bose, Bilateral multiple recession coverage with platelet-rich fibrin in comparison with amniotic membrane, J. Indian Soc. Periodontol. 18 (1) (2014) 102–106.
[103] J. Kaur, S.C. Bathla, Regenerative potential of autologous platelet-rich fibrin with and without amnion membrane in the treatment of Grade-II furcation defects: a clinicoradiographic study, J. Indian Soc. Periodontol. 22 (3) (2018) 235–242.
[104] S.V. Kothiwale, P. Anuroopa, A.L. Gajiwala, A clinical and radiological evaluation of DFDBA with amniotic membrane versus bovine derived xenograft with amniotic membrane in human periodontal grade II furcation defects, Cell Tissue Bank. 10 (4) (2009) 317–326.
[105] K.A. Kumar, et al., Use of an amniotic membrane as a novel barrier in a tooth with a questionable prognosis, J. Indian Soc. Periodontol. 21 (3) (2017) 237–240.
[106] A.C. Mamede, et al., Effect of amniotic membrane proteins in human cancer cell lines: an exploratory study, J. Membr. Biol. 247 (4) (2014) 357–360.
[107] L. Hossain, et al., Human amniotic membrane and its anti-cancer mechanism: a good hope for cancer therapy, SN Compreh. Clin. Med. 1 (7) (2019) 487–495.
[108] M. Magatti, et al., Amniotic membrane-derived cells inhibit proliferation of cancer cell lines by inducing cell cycle arrest, J. Cell Mol. Med. 16 (9) (2012) 2208–2218.
[109] R. Riedel, et al., Human amniotic membrane conditioned medium inhibits proliferation and modulates related microRNAs expression in hepatocarcinoma cells, Sci. Rep. 9 (1) (2019) 14193.
[110] K. Modaresifar, et al., The effect of cryopreservation on anti-cancer activity of human amniotic membrane, Cryobiology 74 (2017) 61–67.
[111] R.G. Alvim, et al., The potential risk of tumor progression after use of dehydrated human amnion/chorion membrane allograft in a positive margin resection model, Therap. Adv. Urol. 11 (2019), 1756287219837771.
[112] S. Bu, et al., Human amniotic epithelial cells inhibit growth of epithelial ovarian cancer cells via TGF-β1-mediated cell cycle arrest, Int. J. Oncol. 51 (5) (2017) 1405–1414.
[113] J. Zhou, T. Liang, D. Wang, et al., IFNα-expressing amniotic fluid-derived mesenchymal stem cells migrate to and suppress HeLa cell-derived tumors in a mouse model, Stem Cell. Int. 2018 (1241323) (2018), https://doi.org/10.1155/2018/1241323.
[114] T.C. Peak, et al., Exosomes secreted by placental stem cells selectively inhibit growth of aggressive prostate cancer cells, Biochem. Biophys. Res. Commun. 499 (4) (2018) 1004–1010.
[115] S. Gholizadeh-Ghaleh Aziz, Z. Fardyazar, M. Pashaiasl, The human amniotic fluid mesenchymal stem cells therapy on, SKOV3, ovarian cancer cell line, Mol Genet Genomic Med 7 (7) (2019), e00726.
[116] S. Sheller, et al., Amnion-epithelial-cell-derived exosomes demonstrate physiologic state of cell under oxidative stress, PloS One 11 (6) (2016), e0157614.
[117] F. Vakhshiteh, F. Atyabi, S.N. Ostad, Mesenchymal stem cell exosomes: a two-edged sword in cancer therapy, Int. J. Nanomed. 14 (2019) 2847–2859.
[118] P. Bourke, et al., Microbiological interactions with cold plasma, J. Appl. Microbiol. 123 (2) (2017) 308–324.
[119] Christl, L., *FDA's Overview Of the Regulatory Guidance For* the Development and Approval of Biosimilar *Products In the US*.
[120] https://www.marketwatch.com/.