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Literature review

Can the amniotic membrane be used to treat peripheral nerve defects? A review of literature

La membrane amniotique peut-elle être utilisée pour traiter les pertes de substances nerveuses périphériques ? Une revue de littérature

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ABSTRACT

Human amniotic membrane is currently being used in ophthalmology and dermatology applications. The objective of this review was to establish proof-of-concept for using amniotic membrane to treat peripheral nerve defects. We performed a search using: 1) PubMed with the keywords/MeSH terms: "amnion", "amniotic membrane", "angiogenesis", "anti-microbial", "characteristic", "chorion", "epithelialization", "fibrosis", "gap", "growth factors", "use", "nerve"; 2) the American clinical trials registry with "amniotic membrane"; 3) Lim Jeremy's book "A primer on amniotic membrane regenerative healing"; 4) the search engine Google. Our findings pointed to the amniotic membrane being a biodegradable and bioactive scaffold that contains many growth factors important for efficient nerve regeneration. Multiple animal studies and the single human clinical trial performed up to now have highlighted its role in preventing recurrence of perineural adhesions, reducing fibrosis, accelerating nerve repair and improving nerve function. Thus, the amniotic membrane has ideal properties for treating peripheral nerve injuries. It could very likely prevent neuroma formation. The best format would be a freeze-dried one containing the amnion and chorion layers in order to preserve all its growth factors, and facilitate its handling and storage in the operating room.

Mots clés :
Anti-fibrosant
Support
Facteurs de croissances
Preuve de concept

RÉSUMÉ

L'objectif consistait à établir une preuve de concept sur l'utilisation de la membrane amniotique humaine, déjà largement exploitée en ophtalmologie et en dermatologie, pour traiter une lésion nerveuse périphérique avec perte de substance. Notre méthode de recherche a utilisé: 1) le site "PubMed" avec comme mots clés/MeSH: "amnion", "amniotic membrane", "angiogenesis", "anti-microbial", "characteristic", "chorion", "epithelialization", "fibrosis", "gap", "growth factors", "use", "nerve"; 2) le site "Clinical trials", avec: "amniotic membrane"; 3) l'ouvrage de Lim Jeremy "A primer on membrane regenerative healing"; 4) le moteur de recherche Google. Les résultats ont montré que la membrane amniotique est un support biodégradable et bioactif possédant de nombreux facteurs de croissance propices à une régénération nerveuse efficace. Les modèles animaux répertoriés et le seul essai clinique ont révélé son action dans la prévention de la récidive des adhérences périneurales, la réduction de la fibrose, l'accélération de la régénération de la structure nerveuse et l'amélioration de la récupération de la fonction nerveuse. Ainsi la membrane amniotique possède des propriétés optimales pour l'indication ciblée et empêcherait très certainement la formation d'un névrome. Son format de prédilection serait une forme lyophilisée, avec ses deux feuillets originels afin de conserver le maximum de facteurs de croissance et faciliter sa manipulation et son stockage au bloc opératoire.

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1. Introduction

Traumatic peripheral nerve lesions are a genuine public health problem as there are more than 300,000 cases per year in Europe [1]. The incidence of finger lacerations with nerve injury is up to 6.2/100,000 inhabitants, resulting in a significant cost to society [2].

Several techniques, such as autologous nerve graft, autograft vein conduits, nerve transfer, collagen or silicone tubes, and neurotubes, have been described for repairing peripheral nerve defects [3]. However, these techniques have drawbacks, [4] such as donor site morbidity for autologous grafts, development of fibrosis and limitations on defect size for neurotubes [5] and vein conduits [6].

One of the goals of peripheral nerve repair is to optimize the healing process in order to prevent neuroma development. Posttraumatic neuromas are a benign, disorganized tissue stemming for an unsuccessful attempt at repairing a peripheral nerve, which results in a "pseudotumor". It appears 1 to 12 months after the injury event. Yülsel et al. believe neuromas develop because of injury to the perineurium [6]. Axons that are not contained in their envelope can escape into adjacent tissues. This phenomenon is accompanied by significant cell proliferation (fibroblasts, Schwann cells) and by blood vessel development. The result is a disorganized network of axons, which are deviated and curled up [7].

In recent years, the amniotic membrane has had tremendous success in repairing soft tissues, particularly in the ophthalmology and dermatology context [8], making it a relevant option in nerve repair. Riccio et al. reported encouraging results in humans for repairing posttraumatic nerve defects 3 to 5 cm in length [9]. This tissue is mainly composed of types I, III, IV and V collagen, laminin, proteoglycans, elastin and fibronectin [10]. It is made up of two layers: the amnion (which is in contact with the amniotic fluid) and the chorion. The amnion is 0.02–0.5 mm thick while the chorion is 1 mm thick. The amnion mainly consists of an epithelium, which is rich in epithelial cells, a basal membrane and a fibroblast layer, which is rich in amniotic mesenchymal stromal cells. The amnion itself is sometimes incorrectly called the "amniotic membrane". The chorion consists of a reticular layer, which is rich in chorionic mesenchymal stem cells, a basal membrane, and a trophoblast, which is rich in trophoblast stem cells.

The amniotic membrane contributes to re-epithelialization and has antifibrotic, anti-inflammatory, antiangiogenic, antimicrobial and antiviral properties [11,12]. It can be used as an allograft because the absence of immunogenicity. Furthermore, it contributes to the healing of wounds and has analgesic properties. Its mechanical properties—viscoelasticity due to its high levels of collagen and elastin—and its high levels of several growth factors [13] make it a biologically active scaffold well-suited to the challenge of nerve repair [4].

We performed a review of literature to establish proof of concept on the use of this highly innovative and promising tissue for treating peripheral nerve defects.

2. Material and methods

2.1. Search strategy

The review of literature encompassed several different sources:

- PubMed using the keywords/MeSH entry terms: "amnion", "amniotic membrane", "angiogenesis", "anti-microbial", "characteristic", "chorion", "epithelialization", "fibrosis", "gap", "growth factors", "use", "nerve". The Boolean operator "and" was used to joint "Amniotic membrane" with "Angiogenesis"; "Antimicrobial"; "Characteristic"; "Epithelialization"; "Fibrosis"; "Growth factors"; "Use"; "Nerve". Additional articles were added after manually reviewing the reference list of all the included publications;
- the United States clinical trials registry (https://clinicaltrials.gov/ct2/search), using the keywords/MeSH entry terms "amniotic membrane" and capturing completed or on-going clinical trials;
- the book by Jeremy Lim: A Primer on Amniotic Membrane Regenerative Healing. MiMedx Group, 2015;
- the Google search engine, mainly to search for marketed products.

One of our research group's prior publications describing the clinical use, patents and marketed products was also consulted [14].

2.2. Selection criteria

For preclinical proof of concept, studies were included if they analyzed amniotic membrane use in animal models of nerve defect or nerve transection.

For clinical trials, studies were included:

- from PubMed if they reported on amniotic membrane use in humans for treating nerve defects.
- from the clinical trials registry when they referred to amniotic membrane use in a rheumatology, orthopedic or trauma context.

In both cases (animal models and clinical trials), only articles in which the amniotic membrane was used as a scaffold were retained. In vitro studies or studies using cells isolated from the tissue were excluded.

For evidence in support of nerve repair/regeneration, the most relevant articles, often the most recent ones and those cited as a reference, were used in our review.

Generally, only articles published in English were retained.

2.3. Screening of studies and data extraction

The article selection and data collection was performed independently by two investigators (MB and FG). The title and abstract were selected based on the following question: Is amniotic membrane use in an animal model or clinical study beneficial for treating nerve defects? An article was evaluated in its entirety if the title and abstract pertained to this screening question. Discrepancies between the two investigators were discussed to select the final articles. Tables were generated and used to collect the relevant information.

3. Results

The PubMed search yielded the following (before 01-OCT-2018):

- amniotic membrane "and" angiogenesis: 85 articles;
- amniotic membrane "and" antimicrobial: 894 articles;
- amniotic membrane "and" characteristic: 178 articles;
- amniotic membrane "and" epithelialization: 237 articles;
- amniotic membrane "and" fibrosis: 348 articles;
- amniotic membrane "and" growth factors: 1566 articles;
- amniotic membrane "and" use: 456 articles;
- amniotic membrane "and" nerve: 204 articles.

The clinical trials registry search (before 01-OCT-2018) yielded 186 studies.

3.1. Amniotic membrane format

The human amniotic membrane, and more specifically the amnion (separated from the chorion), is collected from placenta following Cesarean birth. Tissue from vaginal delivery is not used since it is contaminated by the normal vaginal flora. Several methods exist to store this product: cryopreservation, lyophilization and preservation in dried form [15]. Fresh human amniotic membrane, which is sometimes used in animal models, corresponds to tissue that has not been treated after being collected.

In cryopreserved form, the amniotic membrane is packaged in a solution including a cryoprotectant (glycerol or dimethyl sulfoxide–DMSO) and Dulbecco's modified Eagle's medium. The tissue is then stored at − 80 °C and can be used for up to 1 to 2 years. With this type of preservation, 20% to 50% of the cells survive [16]. This is the benchmark format in current practice and clinical trials [8].

In its lyophilized form, the amniotic membrane is quickly frozen at − 50 to − 80 °C, then freeze-dried in a lyophilizer under vacuum. In some cases, the tissue will undergo pretreatment to destroy any residual viable cells; sterilization by gamma irradiation very often follows the lyophilization step. These two additional processing steps offer new hope for ensuring the safety of amniotic membrane from vaginal delivery, thereby increasing the number of placentas collected. This also allows the allograft to be stored in its original format (two layers). In other cases, the chorion (which is in contact with the maternal tissue) is generally separated from the amnion to avoid risk of contamination by the mother's cells. The resulting "amnion–chorion" product is thicker and stronger than amnion alone. Lastly, lyophilization allows the tissue to be stored at room temperature (e.g. in an operating room).

In its dehydrated form, the amniotic membrane is stored at room temperature in a biosafety cabinet and exposed to air for varying lengths of time (12 to 24 hours). This is usually followed by gamma irradiation for sterilization purposes.

Whether the tissue is used in its fresh, cryopreserved, lyophilized or dehydrated form, it may be subjected to de-epithelialization immediately after being harvested in order to remove the epithelial cell layer.

Table 1 lists the features of the amniotic membrane based on its format and suitability for the target clinical indication.

3.2. Preclinical proof of concept

From the PubMed search and after the inclusion and exclusion criteria were applied, 15 articles on animal models were analyzed and relevant data were extracted [17–31] (Table 2). Among these 15 studies, 10 were published after 2009, evidence of the recent interest in using amniotic membrane for nerve repair. The studies appear to be highly relevant given the number of animals used, the control groups, comparison with other treatments and functional outcomes.

In these animal models, the human amniotic membrane was used in its fresh or cryopreserved form and was very often de-epithelialized. The dehydrated form was used in only two studies [19,31]. In most studies, the model used is one where a localized 1–1.5 cm nerve defect or lesion is made in the sciatic nerve of rats.

The amniotic membrane typically measures 1–2 cm and is secured at each end by an epineural suture [17,19,24–26,28,30,31], photochemical tissue bonding [22,23,27] or is left unsutured [21,29]. Only a few studies specified the orientation of the applied amnion: mesenchymal side against the nerve [21] or, more recently, epithelial side [29,31], after simulation of nerve wrapping *in vitro* [29].

The literature search showed that its use as a perineural wrap appears conducive to repairing a nerve lesion or defect (Table 2). First, this increases the number of axons, nerve fibers and myelinated fibers [18,19,22,25]. For example, for a 1-cm nerve defect, the number of large-diameter axons is higher when an amniotic membrane is used instead of a silicone tube or nerve autograft [18]. It also appears that the diameter of the configured amnion tube greatly affects the repair; a 1–2 mm diameter tube appears ideal [19]. The G-ratio, which is defined as the ratio of the axon diameter to the nerve fiber diameter, is improved [22,25] and the functional recovery, estimated by the Sciatic Functional Index (SFI), is significant [18,25]. From an electrophysiology perspective, Meng et al. showed that amnion combined with nerve autograft helps to increase the amplitude and reduce conduction latency at 8 weeks [25]. Second, the amniotic membrane has a noteworthy effect on fibrosis and perineural adhesions [21,24,25]. Lemke et al. specified that the tissue prevents the recurrence of severe perineural adhesions, reduces perineural and intraneural fibrosis, accelerates nerve structure regeneration and improves recovery of nerve function [29].

Some authors report the benefits of the amniotic membrane in a vascularized form [17], or combined with skeletal muscle [31], hyaluronic acid [21], granulocyte colony stimulating factor (G-CSF) [26] or betamethasone [28] to potentiate its effect. Lastly, photochemical tissue bonding of the amnion compared to fixation using epineural sutures positively affects axon size, axonal migration, nerve fiber diameter, G-ratio index, muscle mass innervated by the transected then repaired nerve, and myelin sheath thickness [22,23,27].

3.3. Clinical trials

A search on the clinical trials registry reveals that more than 100 clinical studies with human amniotic membrane or its derivatives have been performed or are ongoing. However, we did not find any clinical trials that specifically addressed our research question. Thus we decided to compile the 40 clinical trials related to the musculoskeletal system (surgical and medical

Table 1
Summary of the properties of the amniotic membrane in its various formats [8,10–15,35,37–39].

Features	Fresh amniotic membrane	Cryopreserved amniotic membrane	Lyophilized or dehydrated amniotic membrane
Need for cold chain after collection and during handling	Yes	Yes	No
Cell viability	++ (controversial)	+ (controversial)	None
Growth factors	Amnion: ++ Chorion: +++	Amnion: + Chorion: ++	Amnion: + Chorion: ++
Possibility of room temperature storage (e.g. in operating room)	No	No	Yes
Ease of handling and cutting tissue	No	No	Yes
Use in two-layer configuration (amnion + chorion)	No	No	Yes
Additional gamma irradiation	No	Not usually	Yes

+: moderate; ++: high; +++: very high.

Table 2
Use of human amniotic membrane in animal models for repairing nerve defects or nerve transection.

Reference	Animal species (strain)/number	Configuration of human amniotic membrane	Nerve insult/type of nerve	Main findings
Ozcan et al. [17]	Rats (Lewis) 25	Manufactured multilayer amnion tube	1-cm defect Femoral nerve	Vascularized amnion tube achieved nerve regeneration similar to vascularized nerve graft and better than non-vascularized amniotic membrane tube or non-vascularized nerve graft
Mohammad et al. [18]	Rats (Sprague Dawley) 66	De-epithelialized, dehydrated and gamma-sterilized amnion tube	1-cm defect Sciatic nerve	Advantage of amnion tube: Better histological results than with nerve autograft or silicone tube Better function in the early stages
Mligiliche et al. [19]	Rats (Wistar) 24	De-epithelialized and dehydrated amnion tubes of different diameters	0.5-cm defect Sciatic nerve	Advantage of 1–2 mm diameter amnion tube: Same number of myelinated axons as healthy nerve, but smaller in diameter Gastrocnemius muscle is reinnervated after 9 months
Duan et al. [20]	Rats (Wistar) 54	Information not available	1-cm defect Sciatic nerve	Advantage of composite nerve-muscle autografts wrapped with human amnion matrix membrane: More neurofilaments in nerve root; neurofilaments are denser and more regular More axons; axons are larger in diameter and thicker
Ozgenel et al. [21]	Rats (Sprague-Dawley) 72	Fresh de-epithelialized amnion	3-cm defect Sciatic nerve	Nerve wrapping with amnion reduced fibrosis and perineural adhesions This action was bolstered by hyaluronic acid injection
Henry et al. [22]	Rabbits (New Zealand) 24	Cryopreserved amnion	Transection 5 cm from distal end Peroneal nerve	Amnion fixation on nerve using photochemical tissue bonding better than simple suture
O'Neill et al. [23]	Rats (Sprague-Dawley) 24	Cryopreserved amnion	1-cm defect Sciatic nerve	Advantage of photochemical tissue bonding of the amnion over epineural fixation: Better functional outcomes (SFI) at 8 and 12 weeks Greater muscle mass in re-innervated gastrocnemius Larger number of fibers, larger diameter and thicker myelin layer
Kim et al. [24]	Rabbits (New Zealand) 10	Cryopreserved amnion	Transection Ulnar nerve	Nerve wrapping with amnion significantly reduced fibrosis and perineural adhesions compared to contralateral control without wrapping
Meng et al. [25]	Rats (Sprague Dawley) 36	Cryopreserved amnion	1 cm defect Sciatic nerve	Advantage of nerve wrapping with amnion autograft: Improved functional recovery in the early phase after nerve lesion Fewer adhesions and less fibrosis
Fesli et al. [26]	Rats (Wistar) 70	Fresh amnion	Transection Sciatic nerve	Nerve wrapping with amnion reduced perineural fibrosis, improved clinical response and improved electrophysiology data Intraperitoneal GCSF injection potentiated these results
Fairbairn et al. [27]	Rats (Lewis) 100	Cryopreserved de-epithelialized amnion	1.5 cm defect Sciatic nerve	Advantage of nerve wrapping with amnion secured by photochemical tissue bonding versus simple suture or biological glue: Less perineural fibrosis Better SFI results Increased muscle mass in reinnervated gastrocnemius Larger fiber and axon diameter Thicker myelin layer
Sadraie et al. [28]	Rats (Wistar) 42	Fresh de-epithelialized amnion	Transection Sciatic nerve	Advantage of nerve wrapping with amnion combined with betamethasone injection (0.2 mL betamethasone (4 mg/mL)): Better SFI at 4 weeks Improved paw withdrawal reflex (seconds) Reduced conduction latency (ms) Stronger conduction amplitude (mV) More large-diameter (>6 μm) fibers
Lemke et al. [29]	Rats (Sprague Dawley), 54	Fresh amnion with epithelial side placed against nerve	Chemical induction of fibrosis Sciatic nerve	Significant advantage of amnion nerve conduit: Better nerve healing Reduced perineural fibrosis and adhesions Accelerated nerve regeneration: less demyelination, intraneural structure regenerated more quickly, better nerve function (SFI) Regulation of immune response
Hasturk et al. [30]	Rats (Wistar) 25	Cryopreserved human and rat amnion	Transection Sciatic nerve	Advantage of nerve wrapping with amnion allograft over xenograft
Marchesini et al. [31]	Rats (Wistar) 14	Dehydrated amnion filled with skeletal muscle harvested from neighboring tissue	1.5-cm defect Median nerve of forelimb	Experimental support for clinical proof-of-concept [9]

SFI: Sciatic Functional Index; GCSF: granulocyte colony stimulating factor.

Table 3
Clinical trials (completed or on-going) reporting use of the human amniotic membrane in rheumatology, orthopedic and trauma conditions.

Scope of application	Medical condition	Study number (NCT)	Nb. of studies
Musculoskeletal system: Medical treatment	Shoulder osteoarthritis	NCT03770546	1
	Knee osteoarthritis	NCT02318511, NCT02768155, NCT02767492, NCT03074526, NCT03337243, NCT03408145, NCT03441607, NCT03485157	8
	Hip osteoarthritis	NCT03063099	1
	Rotator cuff tear	NCT03379324	1
	Osteochondral lesion	NCT02837484, NCT03036878	2
	Low back pain	NCT02932020, NCT03644251	2
	Tendinitis: Achilles, plantar fascia	NCT01357187, NCT01659827, NCT01996111, NCT02427191, NCT02982226, NCT03414255, NCT03414268	7
	Tendinitis of epicondylar muscles, musculoskeletal problems, trigger finger	NCT01921569, NCT03390920, NCT03583151	3
Musculoskeletal system: Surgical treatment	Hand tendon surgery	NCT02361814, NCT03013582	2
	Ankle tendon surgery: Achilles tendon, fibular tendons	NCT01708187, NCT02719288	2
	Knee surgery: arthroplasty	NCT02088567	1
	Knee surgery: anterior cruciate ligament reconstruction	NCT03294759, NCT03294759	2
	Foot surgery: hallux rigidus	NCT01825356	1
	Spine surgery	NCT02023372, NCT02070484, NCT02300909, NCT02381067, NCT02808234, NCT03113786	6
	Nonunion	NCT03031509	1
		Total studies	40

Source: https://clinicaltrials.gov/ct2/search.

pathology) (Table 3): 25 describe treatments for osteoarthritis, rotator cuff disease, osteochondral lesions, tendinitis or low back pain; 15 relate to tendon lesions in the hand or ankle, anterior cruciate ligament injuries, reducing adhesions during total knee arthroplasty and spinal fusion surgery.

We found a single clinical trial on PubMed describing use of the amniotic membrane as a nerve conduit for treating nerve defects. Riccio et al. reported very promising results in a prospective single-center study performed between March 2012 and September 2013 in the Department of Reconstructive Surgery and Hand Surgery, AOU "Ospedali Riuniti" di Ancona, Italy [9]. The trial involved 5 patients between 22 and 42 years of age (mean of 33.6 years) who had a median nerve injury at the wrist with a 3–5 cm defect (4 cm on average). The time to surgical nerve repair was 0 to 5 months. Fresh or dehydrated amnion was filled with skeletal muscle harvested from an adjacent area. This sheet was then shaped into a tube and secured with 9–0 epineural suture consisting of two stitches at the back and three stitches in front. They found that sensory and motor functions were restored based on the Lister muscular test, Jamar test and Sakellarides classification: one patient had a sensory result of S3, two were S3+ and two were S4; one patient had a motor result of M3 and four had a motor result of M4. This clinical proof of concept was substantiated by a recently published experimental model [31].

The PubMed search also identified a clinical trial that we chose to include in our analysis: treatment of recurrent ulnar nerve syndrome at the elbow in which the patients were treated with an amniotic membrane nerve wrap sutured on itself with 8–0 suture [32]. This retrospective study included 8 patients treated between December 2012 and March 2014. The average pain level on a visual analog scale was lower by 3.5 points after the procedure than before it; the QuickDASH score improved by 30 points, the grip strength by 25 lbs., or 38% compared with the opposite side. Three patients returned to work full time, one patient was able to go from light duty to a full workload, one unemployed patient began a new job and three remained unemployed. No complications were reported.

Nevertheless, this lack of clinical data means that clinical proof of concept studies on amniotic membrane use for treating nerve defects still need to be done.

3.4. Evidence in favor of nerve repair/regeneration

3.4.1. Presence of growth factors

One of the main reasons amniotic membrane is used in tissue engineering is its bioactive nature. In fact, no matter which format is used, this tissue contains a multitude of growth factors [13] including neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin (NT-3), glial-derived neurotrophic factor (GDNF) and ciliary neurotrophic factor (CNTF) [4].

However, certain conditions impact the type and amount of growth factors in the tissue:

- maternal and gestational age: membranes from a full-term pregnancy and from donors under 35 years of age have higher growth factor levels [33];
- harvest site: epidermal growth factor (EGF) concentration can vary two-fold depending on where the amniotic membrane is harvested in the placenta [34]. This variation in concentration may be related to different epithelial cell morphology and different mitochondrial activity depending on the harvest site [35]. A recent study has shown the region opposite the placenta (reflected region) is most suitable for preventing recurrent adhesions and fibrosis [29];
- preservation of both layers (amnion and chorion): the chorion layer contains 82% of the growth factors, except for EGF, of which 20% is contained in the amnion and 80% in the chorion [13,36];
- preservation of epithelium in amnion: fresh amniotic membranes with epithelium are very high in basic fibroblast growth factor (bFGF), EGF, hepatic growth factor receptor (HGFR), keratinocyte growth factor (KGF), keratinocyte growth factor receptor (KGFR), transforming growth factor (TGF) $-\beta 1, 2, 3$ and tumor necrosis factor (TNF) $-\alpha$ [37].

Method of tissue preservation: the fresh form has the most growth factors, followed by the cryopreserved form and then the lyophilized form [38–40]. However, this aspect is controversial in the literature.

Table 4
Summary of the marketed amniotic membrane products, including their composition and features.

Company	Product name	Size	Format/Process	Indications	Similar products
Alliqua Biomedical	Biovance™	1 × 2 to 6 × 6 cm	Decellularized, dehydrated amnion	Acute and chronic wounds (ulcers, burns…) including wounds with exposed tendon, muscle, bone or other vital structures	
AediCell®	Plurivest™ and Demavest™	1.5 × 1; 2 × 3 cm	Freeze-dried and gamma-irradiated placental tissue matrix (placenta disc, amnion/chorion and umbilical cord)	Wounds, ulcers, burns, surgical needs	
AFCell -Liventa Bioscience	AmnioClear™	2 × 2 to 4 × 6 cm	Amnion/Chorion processed aseptically and sterilized	Soft tissue covering, diabetic foot ulcers	
Alon Source Group	ASGBarrier™	2 × 3 to 4 × 8 cm	Wet or dry amnion	Wound healing	ASGFluid™ and AmnioELITE™: amnion and amniotic fluid-derived cells
Alamo Tissue Service	AmnioBioGraft (+)	1–75 sq cm 0.8 to 1.4 cm 2 × 3 to 4 × 8 cm	Amniotic membrane cleaned and sterilized by PASCO2® technology AmnioBioGraft: amnion AmnioBioGraft+: amnion and chorion	Wound healing, ophthalmology	AmnioBioGraft Cord: derived from the umbilical cord
Allosource	Allowrap™	2 × 2 to 4 × 8 cm	Amnion/Chorion cleaned by AlloTrue™ technology Moist (AlloWrap DS) or dry (AlloWrap Dry) configurations	Biological barrier following surgical repair	
Alphatec Spine	AmnioShield	No information	Dehydrated amnion disinfected by Purion℠ process	Chronic wounds, scars	
Amniox Medical	NEOX® 100 and NEOX® CORD 1 K/RT	2 × 2 to 7 × 7 cm 2 × 1 to 8 × 3 cm	NEOX® 100: cryopreserved amnion NEOX® CORD 1 K: cryopreserved umbilical cord + amnion NEOX® CORD RT: room temperature storage NEOX® FLO: lyophilized umbilical cord-based particulate CRYOTEK® preservation process	Wound healing, diabetic foot and chronic ulcers	CLARIX®: reconstruction, tendon repair, nerve decompression, trauma, and arthroplasty RESPINA®: lumbar interbody fusion
Amnio Technology LLC	PalenGen® membrane & hydromembrane	1 × 1 to 8 × 8 cm	Air dried amnion using Pinnacle technologies Dry or wet forms	Wound covering and support for native tissues	XPlus Membrane & XPlus Hydromembrane: wet form providing greater tensile strength, shape manipulation, and slower resorption in vivo PalinGen® Flow: amniotic tissue allografts containing cellular components cytokines, growth factors and extracellular matrix proteins
Applied Biologics	Xwrap™	2 × 2 to 4 × 8 cm	Dehydrated amnion Dry, ECM, Hydro PLUS forms	Wound healing	FloGraft™: cryopreserved injectable amnion
BioDlogics (Integra LifeSciences)	BioDfence® G3	2 × 3 to 10 × 10 cm	Dehydrated, sterilized amnion/chorion stored at room temperature	Wound healing, ophthalmology, nerve, tendon surgery	BioDDryFlex®, BioDOptix®: dehydrated amnion using patent-pending DryFlex® processing technology BioDFence® Sentry™: dehydrated amnion, spongy (intermediate) layer and the chorion BioDFactor®: flowable tissue allograft derived from morselized amniotic tissue and components of the amniotic fluid
Bio-tissue	PROKERA®	No information	Cryopreserved amnion using CryoTek® processing method, held under tension inside a thermoplastic circle	Ophthalmology	PROKERA SLIM, PROKERA, and PROKERA PLUS AmnioGraft® AmnioGuard®
Biocover Laboratories	Amnio-care™	No information	Dry amnion	Ophthalmology	
Celgene Cellular Therapeutics	Acelagraft™	No information	Dehydrated and decellularized amniotic membrane, stored at room temperature	Wound healing, chronic ulcers	

Table 4 (*Continued*)

Company	Product name	Size	Format/Process	Indications	Similar products
Derma Sciences (Integra)	AmnioExcel	Disks: 1.2 to 2.4 cm 1.5 × 1.5 to 10 × 10 cm	Dehydrated using the proprietary DryFlex™ process	Chronic wounds	AMNIOMATRIX®: cryopreserved suspension allograft derived from the amniotic membrane and components of the amniotic fluid using the patented CryoPrime™
Flower Orthopedics™	FlowerPatch™	2 × 2 to 4 × 8 cm	Dehydrated dual layer amnion	Wound healing	FLOWERFLO™: flowable amnion tissue allograft stored at ambient temperatures
IOP Ophthalmics	AmbioDisk™	Disk: 15 mm 1.5 × 2 to 4 × 4 cm	Cleaned, dehydrated and sterilized amnion Visual identification of amniotic membrane orientation	Ocular surface reconstruction	AmbioDry2™: dry state
Keera	AMX: amniotic Membrane eXtract	Soluble powder	Lyophilized extract of the fresh amniotic membrane	Ophthalmology	
MiMedx Group	AmnioFix®	Disk: 16 mm Sheets: 2 × 3 to 4 × 6 cm Wraps: 2 × 2 to 4 × 6 cm	Dehydrated amnion/chorion membrane using Purion℠ process, terminally sterilized	Wound healing, skin substitute product, diabetic foot and venous leg ulcers	EpiFix®, Epiburn® AmnioFill®: minimally manipulated, non-viable cellular tissue matrix allograft AmnioCord®, Epicord®: minimally manipulated, dehydrated, non-viable cellular umbilical cord
Muscoskeletal Transplant Foundation (MTF) biologics	AmnioBand® and AmnioBand® Particulate	Disks: 10 to 18 mm 2 × 2 to 7 × 7 cm Particulate: 40 to 80 mg	Dehydrated sterilized amnion/chorion	Acute or chronic wound covering	AmnioBand® Viable Membrane: cryopreserved allograft
NHS Blood and Transplant	Amniotic membrane small or large	2 × 2 or 3 × 3 cm	Cryopreserved amnion at a minimum of −40 °C in 50% glycerol/50% Hanks solution	Ophthalmic surgery, primarily ophthalmic surface dressing	
Organogenesis	Affinity®	No information	The only fresh hypothermically stored amnion with viable cells (including stromal cells, fibroblasts, and epithelial cells), growth factors and cytokines; processed by AlloFresh® method	Wound healing, soft tissue repairs	Nushield®: dehydrated sterilized amnion/chorion processed by BioLoc™ method (tendons and nerves) NuCel®: cryopreserved amniotic membrane with cells from amniotic fluid; powder in suspension (orthopedic procedures)
Orthofix (MTF)	Versashield®	2 × 2 to 4 × 6 cm 2 × 3; 3 × 8 cm	Dehydrated sterilized amnion/chorion	General surgery, corneal, plastic surgery, burn and wound care, sports foot and ankle procedures, spine and dura repair	
Osiris Therapeutics	Stavux®	2 × 4 to 3 × 6 cm	Cryopreserved human placental tissue composed of umbilical amnion and Wharton's jelly	Tendon repair, achilles tendon rupture, bunionectomy, hallux rigidus correction, foot amputations, fibromatosis, arthrodesis	Graftix®: cryopreserved amnion/chorion (wound healing, Bone and tendon) GrafixPLPime®: lyophilized amnion/chorion
Seedbiotech	Aril®	Disks: 5 to 15 mm Ellipse: 1 × 2 to 8 × 10 cm	Decellularized and gamma irradiated amnion	Orthopedic, surgical, ophthalmology, spinal and wound covering applications	
SurgiLogix	SX Barrier™	2 × 3 to 4 × 8 cm	Wet or dry amnion	Wound healing, tissue repair	AmnioELITE: amniotic fluid allograft comprised of amnion SXFluid™: amniotic tissue allograft comprised of ground amnion and amniotic fluid-derived components
Snoasis Medical	BioXclude®	0.8 × 0.8 to 2 × 3 cm	Washed, dehydrated and sterilized amnion/chorion using Purion℠ process	Dental and maxillofacial surgery, ophthalmology	
SKY orthobiologics	ActiveBarrier® 45, 200, 200	Various thicknesses: ActiveBarrier® 45: 45 μm ActiveBarrier® 200: 200 μm ActiveBarrier® 2000: 2000 μm 2 × 2 to 4 × 8 cm	ActiveBarrier® 45: Amnion ActiveBarrier® 200: Chorion ActiveBarrier® 2000: entire placental membrane ActiveBarrier® 45 & 200: Dehydrated, HydraTek® process ActiveBarrier® 2000: fresh frozen	Chronic wounds, nerve conduit, nerve defect, tendon sleeve	OculoMatrix® & VisiDisc® Thin/WoundEx®45: dehydrated amnion OculoMatrix® & VisiDisc® Thick/WoundEx®200: dehydrated chorion ReVive™ Membrane: dehydrated placental membrane

Table 4 (*Continued*)

Company	Product name	Size	Format/Process	Indications	Similar products
Vivex Biomedical	Cygnus®	Various sizes 1 × 1 to 1 × 12 cm	Dehydrated allograft Cygnus® Solo (amnion) Cygnus® Dual (dual layer amnion) Cygnus® Matrix (amnion/chorion) Cygnus® Max (umbilical cord)	Spine & neurosurgery, foot & ankle, wound care burn care, dermatology, ophthalmology, oral surgery	AlloGen® (frozen or liquid): all-natural liquid matrix allograft derived from amniotic fluid

3.4.2. Antifibrotic, anti-inflammatory and pro- or antiangiogenic properties

These growth factors have specific functions. The antifibrotic effect is one of the primary properties that ensures good nerve healing. Fibroblasts are naturally involved in healing and are activated during the process by TGF-β. The antifibrotic effect of the amniotic membrane is achieved by suppression of TGF-β signaling and reduction in the expression of TGF-β1, 2 and 3 isoforms and TGF-β receptor II [15]. These antifibrotic properties have been demonstrated in various animal models during peripheral nerve repair (Table 2).

The anti-inflammatory effect of the amniotic membrane is guided by inhibition of the expression of proinflammatory cytokines such as bFGF, interferon (IFN) -c, interleukin (IL) -1α, 2, 8, 10, platelet derived growth factor (PDGF) and TNF-β on the damaged ocular surface [15].

Lastly, the tissue's mesenchyme has fibroblasts that synthesize proangiogenic growth factors: angiopoietin (Ang) -2, bFGF, EGF, heparin binding epidermal growth factor (HB-EGF), HGF, PDGF-BB, placental growth factor (PLGF) and vascular epidermal growth factor (VEGF) [41]. These trigger proliferation of endothelial cells and guide the formation of new blood vessels. Paradoxically, the amniotic membrane can also have an antiangiogenic effect [42] through the production of other factors such as tissue inhibitor of metalloproteinase (TIMP) -1, 2 or pigment epithelium-derived factor (PEDF) [42,43]. The microenvironment in which the allograft is implanted will drive the pathway toward pro- or antiangiogenic activity.

3.4.3. Semipermeable and absorbable scaffold properties

Whether the amniotic membrane is used in its cryopreserved or lyophilized form, it is an excellent biodegradable scaffold with biochemical and mechanical proprieties that make it well-suited to nerve regeneration. It has unique nerve conduit capacities because of its structural properties and specific protein elements such as collagen, laminin and proteoglycans. This tissue has been exploited in patents and marketed products [8,16] (Table 4). It has other specific characteristics that are similar to another human tissue type—the induced membrane—and can function as a bioreactor [44].

As for its degradation, the amniotic membrane (depending on the format used) is still visible when animals are euthanized at 4 weeks [29], 6 weeks [45] and 12 weeks [21,23] after its implantation, but is no longer visible at 4 months (16 weeks) [18]. Lemke et al. point out that the membrane can no longer be differentiated from neighboring tissues at 3 months [29]. Thus, beyond this point in time, we cannot expect the tissue to have a positive impact, although it no longer matters, given the speed of the adhesion process in their study.

4. Discussion

There is little data on using the amniotic membrane to repair peripheral nerve defects. This literature review describes the exceptional ability of this tissue to regenerate nerves and/or reduce adhesions and fibrosis and serves as proof of concept for the targeted clinical indication. It supplements the similar evaluation done for oral surgery [46].

As shown in our review's findings, in addition to its fundamental antifibrotic nature, this bioactive tissue has several growth factors and angiogenic factors that can effectively guide nerve regeneration. In fact, certain growth factors such as NGF, BDNF, NT-3 and TGF-β are involved in the formation of fibrosis [47]. For example, TGF-β is produced by cells recruited to the site of the local damage. It converts fibroblasts into myofibroblasts to repair the extracellular matrix components. It also regulates the production of enzymes such as matrix metalloproteinases (MMPs) that catalyze the degradation of extracellular matrix. In the case of excessive scarring, myofibroblasts synthesize an abnormally high amount of extracellular matrix components and MMPs [48]. The amniotic membrane contributes to the synthesis of MMP inhibitors, TIMP − 1, − 2, − 3 and − 4 [49], and has an anti-inflammatory action through modulation of cytokines such as IL-6, IL-10 IL-1β, IL-9, IL-17a, IL-22, TNFα, IFNγ and prostaglandins [50,51]. In animal models, xenografting of this tissue helps to reduce fibrosis in various contexts: nerve lesions [25], burns and chronic wounds [52], peritoneal [53] or uterine horn adhesions [54] and tendon repair surgery [55]. These properties allow the amniotic membrane to act as a scaffold for the development of organized perineural connective tissue, which is a fundamental requirement for good quality axon regeneration.

Our review also highlighted the scaffold properties of this human allograft, particularly strength and resorption, which are compatible with the target clinical indication. While the amnion is no longer clearly distinguishable from other tissues 3–4 months after its implantation in animal models, its resorption rate does not seem to interfere with nerve repair. For clinical use, we theoretically will need to master the physical stability of the membrane so it can prevent adhesion from recurring over time. Along the same lines, we cannot expect consistent production of growth factors by amniotic cells but rather early release of these factors immediately after implantation, from the cells or matrix being degraded [29]. We propose using a multilayer product (superimposition of several membranes) and/or the original format (both layers: amnion and chorion) to increase its thickness and thus slow its resorption rate to meet this physical stability requirement.

The last element is that this tissue has very little immunogenicity based on the HLA (human leukocyte antigen) system [56]. On one hand, it has been stated that an allograft of amniotic membrane in animals provides a better environment for nerve regeneration than a xenograft [30]. On the other hand, the foreign body reaction is minimal when fresh human amniotic membrane xenograft is used in animal models [29,30]. Thus it is not much of a leap to think that using this tissue will induce little to no foreign body reaction as an allograft in humans. This will also reduce the likelihood of making the fibrosis worse, which sometimes happens when slightly immunogenic neurotubes are used.

5. Conclusion

The use of amniotic membrane to repair peripheral nerve defects appears very promising based on in vivo animal studies and very early clinical data. This tissue has inherent properties such as bioactivity, immunotolerance and antifibrotic effect that we believe, based on extrapolation, to be very likely to prevent neuroma formation. With the appropriate regulatory approvals, this scaffold could be used in humans, preferably in the lyophilized form consisting of the two original layers (amnion, chorion). The goals would be to:

- preserve as many growth factors as possible;
- make it easy to handle and store at room temperature in the operating room;
- slow down its resorption rate.

Its lyophilized form, often supplemented with a virus inactivation process, is theoretically a safer product. Its optimal format appears to be as a tubular membrane, possibly in multiple layers, secured by epineural sutures at both two ends of the nerve defect.

Disclosure of interest

The authors declare that they have no competing interest.

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