SALVIN® Dental Specialties

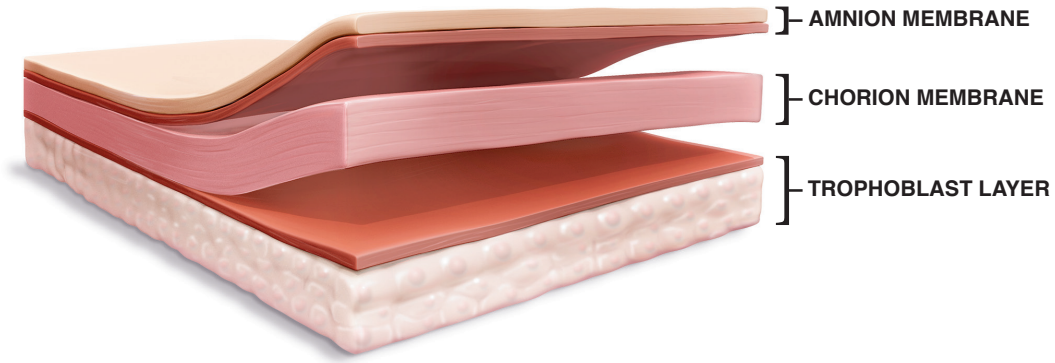
SALVIN® Regenerative

NEW!

SALVIN® AMNIO EXCITE®

Amnion / Chorion Membrane

Structure of Salvin AmnioExcite® 3-Layer Placental Membrane



AmnioExcite® Includes Both Amnion And Chorion PLUS The Trophoblast Layer

The Trophoblast Layer Contains More Than 50% Of The Biologically Relevant Proteins In The Placental Membrane (1)

- 4X Thicker Than An Amnion Or Chorion Membrane For A Substantial Protective Barrier (2)
- Does Not Fold In Upon Itself When Rehydrated, Making It Much Easier To Handle
- LifeNet Health®'s Matracell® Decellularization Process Serves To Minimize Immunogenic And Inflammatory Responses (3,4)

SALVIN® AMNIO EXCITE® **Amnio / Chorion Membrane**

Size	Product #	Fee	7 or More
10mm x 10mm	#AMNIO-MEM-10X10	89.00	80.00
15mm x 20mm	#AMNIO-MEM-15X20	169.00	157.00
20mm x 30mm	#AMNIO-MEM-20X30	259.00	239.00

1. *LifeNet Health. TR-004-2020 Characterization of the Amnion, Chorion, and Trophoblast Layers of Decellularized and Freeze-Dried Placental Membrane. 2020.*
2. *Verbruggen SW, Oyen ML, Phillips AT, Nowlan NC. Function and failure of the fetal membrane: Modelling the mechanics of the chorion and amnion.PLoS One. 2017; 12(3):e0171588*
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4. *Moore MA, Samsell B, Wallis G, Triplett S, Chen S, Jones AL, et al. Decellularization of human dermis using non-denaturing anionic detergent and endonuclease: a review. Cell Tissue Bank. 2015;16(2):249-59.*

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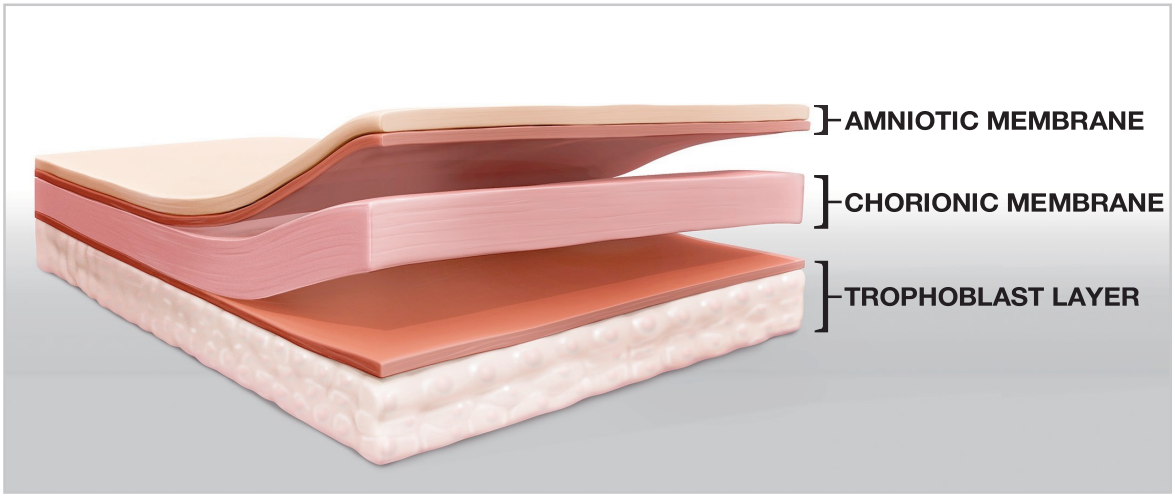
The Science of AmnioExcite® Placental Membrane Allograft

AmnioExcite® is a full-thickness *decellularized* placental membrane.

AmnioExcite® is a lyophilized, full-thickness placental membrane allograft decellularized with LifeNet Health's proprietary Matracell® process and patent pending technology and intended for homologous use as a barrier membrane.(1) Inclusion of the intact amniotic and chorionic membranes, as well as the trophoblast layer, makes it thicker than most available amniotic-only or amniotic-chorionic allografts, and provides a robust protective covering while also delivering superior handling. AmnioExcite® retains the placental membrane's naturally occurring growth factors, cytokines, protease inhibitors, and extracellular matrix components, such as proteoglycans, collagen and fibronectin(2) *In vitro* studies have shown that these endogenous factors are capable of inducing cellular proliferation and migration, mitigating inflammation, and inhibiting protein degradation(3-5)

STRUCTURE OF THE THREE LAYER PLACENTAL MEMBRANE



The placental membrane is comprised of the amnion and chorion(6). The amnion, also called amniotic membrane (AM) has five layers, including the epithelium, basement membrane, compact layer, fibroblast layer, and the spongy layer(6), which provide important extracellular membrane components, as well as a wide variety of growth factors, cytokines, and other proteins.(7) While these characteristics are important, the AM by itself lacks substantial structure for providing a protective covering and contains only a small portion of the biological factors found in the full-thickness placental membrane. AM-only grafts can also be difficult to apply and may migrate away from the intended site of application.(8)

The chorion is comprised of four layers, including the cellular layer, reticular layer, the pseudobasement membrane and the trophoblast layer (TL) (6). The cellular layer, reticular layer, and the pseudobasement membrane can be separated from the trophoblast layer; these three layers together can be referred to as the chorionic membrane (CM), which contains many of the same growth factors as the AM.

The trophoblast layer (TL) is approximately four times thicker than the AM or the CM (Figure 1). Its thickness provides for a more substantial protective barrier, which may provide a protective environment by preventing fluid loss as well as bacterial ingress.(9) The trophoblast layer also contains the majority of biological factors in the placental membrane.(10) Unlike other AM-only or AM-CM membrane allografts, the layers in AmnioExcite® are never separated during the entire process because separation can adversely impact retention of the native growth factors.(1) Therefore, AmnioExcite® retains more endogenous growth factors than similar placenta-derived allografts by being processed as an intact, whole placental membrane.

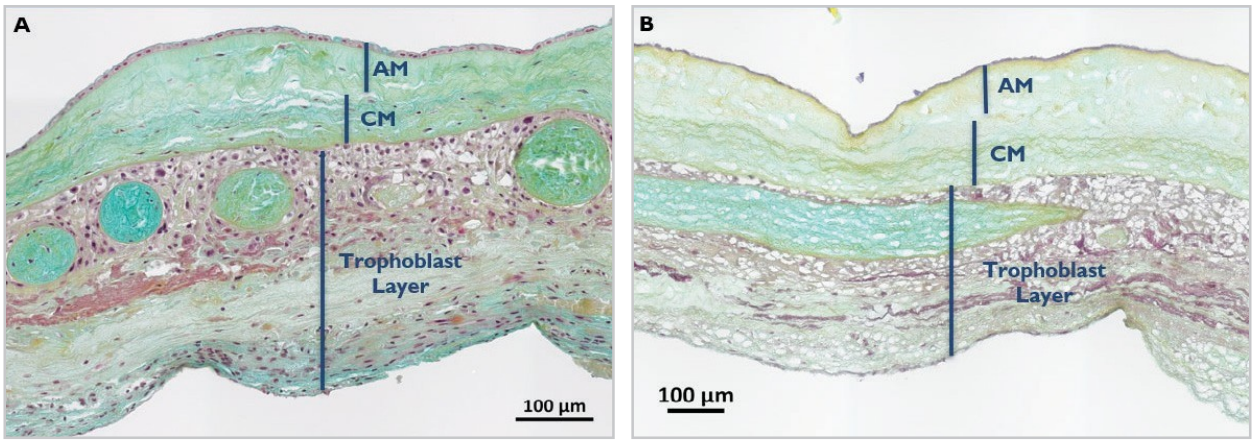


Figure 1. Decellularization of the Placental Membrane. A) Native placental membrane prior to decellularization. B) Matracell-processed decellularized placental membrane including the trophoblast layer. Russell-Movat Pentachrome staining shows the collagen (yellow) and glycosaminoglycans (GAGs; green). The dense darker line on the surface shows the basement membrane layer. The chorionic membrane (CM) has variable thickness, but is thicker than amniotic layer (AM) and appears as different shades of green, indicating proteoglycans (GAGs). The trophoblast layer (TL) is the thickest layer, and thickness varies from 200 – 600 µm. Scarce remnants of cellular cytoplasm are stained dark red. Elastin fibers are stained dark gray/purple and located mostly within this layer. Note the porous nature of the trophoblast layer after decellularization. (10-13)

Benefits of *Full-thickness*, *Decellularized* Placental Membrane

Full-thickness: Superior barrier and handling

Due to the thickness of the trophoblast layer, AmnioExcite® does not fold in upon itself when rehydrated, making it less likely to migrate away from its intended site of application and ensuring full coverage of the wound site as intended.(14) AmnioExcite®'s unique thickness also provides a robust natural barrier that may help prevent bacterial ingress and thus inflammation associated with infection.

Decellularization: Reduced immunogenicity and increased porosity

In most placental-based allografts, the trophoblast layer is removed. However, by removing this layer, a rich source of beneficial endogenous biological factors and thick structural matrix are also removed. A few tissue processors do retain the trophoblast layer without removing non-viable cells, which may provoke an immune response due to the presence of antigens on the cells.(15) Additionally, the recipient's immune system will need to remove these dead cells, which may result in an excessive inflammatory response.(16) Although some inflammation is a natural part of wound healing, excessive or chronic inflammation, such as seen in chronic wounds, impedes healing.(17) The ideal solution is to leave the thick, nutrient rich extracellular matrix of the trophoblast layer intact but remove the cells to avoid an immune or inflammatory response.

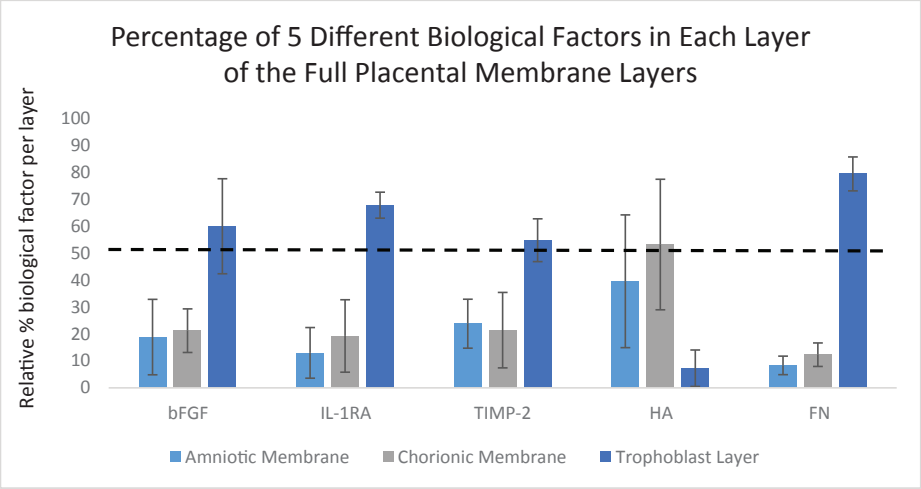
LifeNet Health has solved this problem by using the patented Matracell decellularization process to remove potentially immunogenic cells while retaining the trophoblast layer's rich concentration of growth factors and thick extracellular matrix. (18, 19) The Matracell decellularization process removes substantial amount donor cells (> 90% cell removal). The sum of the DNA content in AmnioExcite® is lower than 10ng/mg of wet weight. (10) Additionally, the decellularization process creates a more porous trophoblast layer, which is unique to AmnioExcite®. Porosity is an important membrane characteristic that can affect access to nutrients, waste removal, cell migration, and angiogenesis.(20) *In vitro* experiments have found that substrate density appears to inversely correlate with neovascular growth rate, such that a porous matrix encourages a higher rate of vascularization.(21) Porosity also increases surface area, which may be beneficial for cellular migration. (20, 22, 23). AmnioExcite®'s enhanced porosity makes it a uniquely biohospitable scaffold for nutrient exchange, cell migration, and neovascularization.

Factors:

When the trophoblast layer is removed, many endogenous proteins are lost.(10) AmnioExcite® is the only placental membrane allograft to include a decellularized trophoblast layer. MagPix or ELISA quantification, which measures the presence of specific proteins, shows that AmnioExcite® maintains the growth factors and extracellular matrix components known to be inherent to placental tissue (Table 1). Additionally, by maintaining the trophoblast layer, which is frequently removed in other placental-based allografts, AmnioExcite® retains those endogenous growth factors. In a study measuring the concentrations of bFGF, IL-1RA, TIMP-2, hyaluronic acid, and fibronectin in the AM, CM, and the trophoblast layer, the TL accounts for more than 50% of endogenous growth factors for all but hyaluronic acid, suggesting that in grafts in which the trophoblast layer is removed, potentially more than half of the endogenous factors in the placental membrane are lost (Figure 4). (10) Table 1.

Table 1. Relevant biological factors present in full thickness placental membrane(2)

	Abbreviation	Name
Growth Factors and Cytokines	TGF-β 1	transforming growth factor beta 1
	TGF-β 3	transforming growth factor beta 3
	PDGF-BB	platelet derived growth factor subunit B
	ANG	Angiogenin
	EGF	epidermal growth factor
	bFGF/FGF-2	fibroblast growth factor 2/basic
	VEGF-A	vascular endothelial growth factor A
	PIGF/PGF	placental growth factor
	HGF	hepatocyte growth factor
	IL-1RA	interleukin 1 receptor antagonist
	PTX-3	pentraxin 3 (aka TSG-14)
Protease Inhibitors	TIMP 1	tissue inhibitor of metalloproteinases 1
	TIMP 2	tissue inhibitor of metalloproteinases 2
	TIMP 3	tissue inhibitor of metalloproteinases 3
	TIMP 3	tissue inhibitor of metalloproteinases 4
	A2M	alpha-2-macroglobulin
Extracellular Matrix	HA	hyaluronic acid (aka hyaluronan)
	PRG-4	proteoglycan 4; lubricin
	Fibronectin	Fibronectin



The Trophoblast Layer Accounted For More Than 50% Of 4 Out Of 5 Biological Factors.

Figure 4. Relative quantities of biological factors in the three placental membrane layers. The amount of each biological factor and ECM component was measured and calculated as picogram or nanogram per mm^2 for each layer. The total quantity for each biological factor was the sum of all layers per mm^2. The quantity per layer was divided by the total quantity and multiplied by 100 to determine the percentage of each biological factor in either the AM, CM, or the TL alone. The percentages of each biological factor and ECM component quantified are shown as an average of the 3 donors tested. (10)

One particular protein concentrated in the trophoblast layer is fibronectin. In AmnioExcite®, approximately 80% of the fibronectin was found in the retained trophoblast layer. *In vitro* studies have found that fibronectin is important for cell migration and attachment to the extracellular membrane as well as cell-to-extracellular matrix communication, a process known as dynamic reciprocity; however, *in vitro* results may not be generalizable to performance in humans.(3, 17) AM-only or AM-CM grafts may lose up to 80% of the fibronectin available in the entire placental membrane as well as up to 50% of other important proteins, such as bFGF, IL-1RA, and TIMP-2.(2) Apart from hyaluronic acid, which was concentrated in the AM and CM, the trophoblast layer appears to hold a majority of the proteins and growth factors in the full-thickness placental membrane.

Conclusions

AmnioExcite® is the only placental membrane allograft that retains the trophoblast layer which houses more than 50% of the biologically relevant proteins in the placental membrane, while also avoiding immunogenic and excessive inflammatory responses through decellularization. By including a decellularized trophoblast layer, AmnioExcite® provides superior handling, a porous biohospitable scaffold for cellular migration and attachment, and retains more than 50% of biological factors and extracellular matrix components endogenous to the placental membrane.

Matracell: The decellularization science behind AmnioExcite®

AmnioExcite® is decellularized using the propriety Matracell process, which yields a biohospitable scaffold designed to avoid excessive inflammatory response. Matracell is a patented and validated process that renders allograft tissue acellular, without compromising the biomechanical or desired biochemical properties of the allograft bio-implant for its intended surgical application.(18) Further, Matracell processed tissues support cell migration, cell proliferation, and vascularization.(24, 25) AmnioExcite® is thoroughly decellularized, while also retaining relevant extracellular matrix components, growth factors, and other proteins.(2, 10)

The Matracell process for AmnioExcite® involves four steps:

Step One – Decellularization. Matracell utilizes an anionic, non-denaturing detergent, N-Lauroyl sarcosinate, to remove donor cells from the allograft bio-implant. N-Lauroyl sarcosinate is a common detergent used widely in the cosmetic industry due to its bactericidal properties and safety profile. To remove the donor nucleic acids, a recombinant endonuclease is used to efficiently degrade DNA and RNA without introducing the risk of prion disease transmission. The process is also designed to degrade DNA and RNA-based viruses, contributing to virus inactivation. The entire placental membrane is processed in an intact state using patent-pending technology.

Step Two – Rinsing. Decellularization reagent residuals and donor cell remnants are removed from the allograft through a series of rinsing steps, leaving AmnioExcite® free from unwanted residues.

Step Three – Preservation. The full thickness, decellularized placental membrane is lyophilized, which allows for storage at room temperature. The final moisture content is less than 15%.

Step Four – Sterilization. This final step involves the use of low-dose gamma irradiation performed at ultra- low temperatures. The final allograft has a Sterility Assurance Level (SAL) of 10^{-6}, which is the recommended SAL for medical devices or allografts that are implanted or come in contact with breeched skin.

LifeNet Health's Exemplary Safety Record

LifeNet Health has been providing allograft tissues for clinical use since the 1980s, establishing an unparalleled record of safety while revolutionizing allograft preparation. Since 1995, LifeNet Health has distributed more than seven million implants cleaned with our patented Allowash technology, without a single case of disease transmission.

Before tissue preparation is even initiated, LifeNet Health goes beyond industry standards with rigorous donor screening, stringent tissue-recovery protocols, and testing of all recovered tissue for bacteria and fungi. Additionally, state-of-the-art nucleic acid testing is performed to detect the presence of specific viruses in donor blood samples. Together, these steps minimize the chance that infected tissue would even be processed. As further assurance, a physician medical director assesses all medical and testing records to affirm that each donor's gifts are safe for transplant.

LifeNet Health applies a range of processing techniques that are customized to each tissue type to mitigate the risk of viral and bacterial disease transmission. This includes their Matracell decellularization technology, which has been designed to degrade a virus's genetic material and dissolve its protective envelope, allowing the virus's remnant fragments to be removed from tissue along with the donor's cells.

Finally, LifeNet Health's terminal sterilization process renders AmnioExcite® sterile to Sterility Assurance Level (SAL) 10^{-6}, which is recommended for surgical implants. In fact, studies have shown that our processes are robust enough to inactivate DNA, RNA, enveloped and non-enveloped viruses.(26)

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This technical monograph was developed by LifeNet Health®

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