Characterization of Minimally Processed Placental Umbilical Cord Membranes Developed for use as a Surgical Implant Around Peripheral Nerve

Curt Deister, PhD, Stefanie Marquez-Vilendrer, PhD, Anne Engemann, PhD, Brian Romot, MS and Erick DeVinney, BS, AxoGen Corporation, Alachua, FL

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Introduction

Inflammation is a normal component of wound healing and can result in increased scar formation and adhesions. Placental tissues have been studied for their potential for reducing inflammation and adhesion formation, but their use as an implant for internal surgical applications has been limited by their mechanical properties and longevity. Processed umbilical cord membrane that retains the beneficial placental properties of the tissue and exhibit ideal mechanical properties may be appropriate for a surgical implant. This work describes the evaluation of processed placental membranes, isolated from human umbilical cord membrane for mechanical strength, resorption, and preservation of inherent tissue properties.

Methods

Minimally processed, human dehydrated umbilical cord membrane (dUCM) and human dehydrated amnion-chorion (dHACM) samples were used. For mechanical testing, samples were hydrated in isotonic saline for 5-20 min prior to testing.

Suture pullout testing: 6-0 suture placed with bite depth of ~3-6 mm. Pull rate was set at 25.4 mm/min and conducted to failure. Maximum force and failure mode were recorded.

Tensile strength testing: Samples were clamped into pneumatic side action grips with pressure between 40 and 60 psi and gage length of 5mm. Pull rate was set at 25.4 mm/min and conducted to failure.

Methods Cont.

Thickness measurement: Measurements were made using a Mitotoyo push gauge with ball-anvil, accuracy +/-0.025mm with 0.01mm increments. For each sample, three measurements were averaged. The ball-anvil avoided errors from folds in the tissue, but compresses the tissue.

In Vivo resorption study: *In vivo* resorption was investigated by implantation subcutaneously in Sprague-Dawley rats, using 1x1cm samples. Samples were examined macroscopically and histologically at 4, 8, 13, and 16 weeks (n=16 samples per point).

Growth factor level determination:
A Luminex® 200™ (Luminex Corporation) with commercial Millipore kits was used. Samples were prepared, briefly, by homogenizing, extracting with 8M guanidine, centrifuging, and dialyzing (Ref 1).

Results

Suture pullout testing:

~3 fold greater retention strength in dUCM

Sample	n	Maximum Force (N)			
		Mean	SD	Min	Max
dUCM	10	1.47*	0.58	0.54	2.13
dHACM	6	0.45	0.27	0.20	0.74

* significant difference p<0.01; all failed by pull-out

Tensile strength testing:

Sample	n	Maximum Force (N)			
		Mean	SD	Min	Max
dUCM*	26**	12.08	3.63	4.21	19.46

* 1x2 cm samples tested along long axis

** 24 samples failed within the tissue, 2 samples failed at the grips

Results Cont.

Thickness measurement:

~2.5 fold greater thickness in dUCM;

>8 fold thicker than single layer amnion, when amnion is 20-25% of dHACM thickness (Ref 2).

Sample	n	Dry Maximum Thickness (µm)			
		Mean	SD	Min	Max
dUCM	18	77*	32	30	163
dHACM	10	29	5	23	40

* significant difference p<0.01

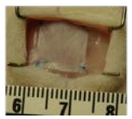
Sample	n	Dry Maximum Thickness (µm), non-compressive*			
		Mean	SD	Min	Max
dUCM	26	107	35	55	191

* using ~5mm diameter flat anvil head with ~2N force

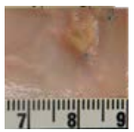
In Vivo resorption study:

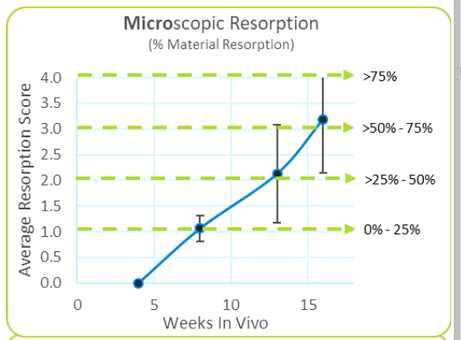
Grafts are present for at least 16 weeks and progressively resorb during that time.

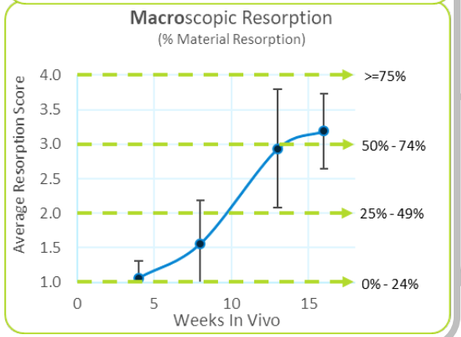
T=0



T=16 week



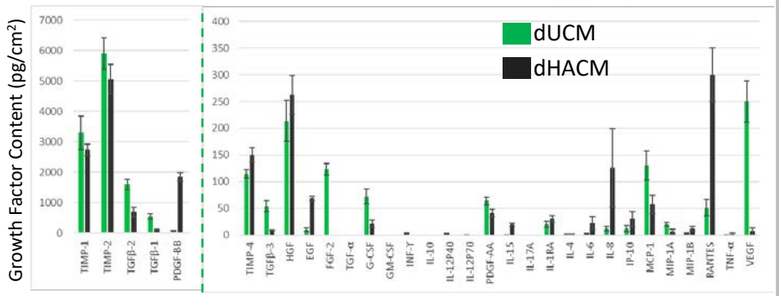




Results Cont.

Growth factor level determination:

27 growth factors were detected in dUCM, as compared to 23 in dHACM, from a panel of 31 growth factors. Error bars are SEM.



Higher levels of TGF-β1, TGF-β2, TGF-β3, FGF-2, and VEGF were found in dUCM. dHACM showed higher levels of PDGF-BB, EGF, IL-8, and RANTES.

Conclusions

- Human dehydrated umbilical cord membrane provides characteristics and properties appropriate for a surgical implant
- Human dUCM grafts are thicker and stronger than other placental membranes, and have ideal handling properties and sufficient strength to secure the graft surgically
- Grafts remain present for at least 16 weeks, relevant to the critical phase of tissue repair, clinical inflammation, and fibrosis
- Growth factors inherent to placental tissues, such as TGF-βs, FGF-2, and VEGF, were detected in dUCM

References

1. (Modified from) Cooke, M., Tan, E.K., Mandrycky, C., He, H., O'Connell, J. and Tseng, S.C.G. Comparison of cryopreserved amniotic membrane and umbilical cord tissue with dehydrated amniotic membrane/chorion tissue. J Wound Care 2014 Oct; 23(10): 465-74.
2. Koob, T. J., Lim, J. J., Massee, M., Zabek, N. & Denozière, G. Properties of dehydrated human amnion/chorion composite grafts: Implications for wound repair and soft tissue regeneration. J. Biomed. Mater. Res. B Appl. Biomater. 102, 1353–1362 (2014).