Appendix P

Clinical literature review: Application of dehydrated human amnion/chorion membrane (dHACM) products for wound healing and surgical applications.

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Introduction

Limited literature currently specifically exists for the use of dehydrated whole placental membrane for clinical use. However, a substantial body of literature exists for the clinical use of amnion, chorion or amnion/chorion composite material in both dehydrated, cryopreserved and glycine preserved formats (Silini *et al.* 2015). Previously, the use of human amniotic membrane allografts for the treatment of wounds, for example, was limited due to issues surrounding the sourcing, preparation and risk of infectious disease transfer. More recently, advances in amniotic tissue preparation, stabilisation of biological activity and storage have resulted in 'off-the-shelf' allograft products for therapeutic use (Silini *et al.* 2015). The reader is referred to the attached document "Bibliography of Amniotic Technology" for a list of additional reading which will give a broader overview of studies using amniotic membrane for a range of medical purposes (Stimlabs 2016). This review will focus on the benefits and risks of using dehydrated placental derived allografts in wound care and surgical applications specifically with reference to a limited number of case studies performed with a relatively new amniotic membrane product called Revita® and in the context of its intended use i.e. for utilisation in wound repair and various surgical applications.

Brief review of Revita®

Revita®, produced by Stimlabs in Atlanta, USA and proposed to be produced under licence by Surgical Biofix in Brisbane Australia, consists of dehydrated placental membrane. The reader is referred to a brief review of the rationale for the development of Revita, it's production process and the unmet clinical need that it supports (Stimlabs 2018). The review also summarises six case studies of 1 venous leg ulcer (VLU), 4 diabetic foot ulcers (DFUs) and a surgical wound. Rather than detail these here the reader is also referred to the attached report which briefly summarises the clinical histories, treatment regimen and time course to healing of each wound. However, the report does not provide a concluding summary therefore one is provided here based on the interpretation of the available data by this reviewer. It appears from the data presented that Revita was well tolerated by each of the patients, was safe and did not result in infection or any reported adverse events. The efficacy of Revita appears similar in terms of initiating healing responses in chronic non-healing wounds over similar time scales to other dehydrated amniotic membrane products (reviewed below). However, it is clear that additional case studies and in particular large RCTs would better assist with determination of actual efficacy of Revita in this clinical situations. Importantly, the self-report data clearly indicates that the treating clinicians rated the handling properties of Revita as excellent in all cases which is a clear

feature of the manufacturing process. While there is no inference of bias in the report, it was obviously produced by Stimlabs and as such there is a clear COI.

Literature search and review strategy

Since Revita is a dehydrated amniotic membrane product which is new to both clinical application and therefore the wound care and surgical markets, the following clinical review will largely focus on similar 'first-generation' dehydrated amnion, chorion and amnion/chorion composite allograft materials.

An initial search of the CINAHL, Chochrane, Medline and Pubmed literature databases was conducted using combinations of the search terms Wound*, Amnion*, Chorion*, and Allograft (Table 1). Collectively, this resulted in the retrieval of 873 publication records which were deposited into a dedicated Endnote library. Following removal of duplicates 766 records were retained for further filtering based on the inclusion of records that only contained the search (Amnion* OR Chorion*) AND Allograft, which resulted in 332 identified records. An additional round of filtering to only include titles that included the word 'dehydrated' and excluded titles that contained the word 'micronised' resulted in an initial 35 publication records for review (Table 1). Of these one publication that involved *in vitro* examination of stem cell activity was also excluded from review. During the review process a few additional publications were cited where points of particular interest were found to be relevant to the point of the topic or to provide broader context.

Table 1. Literature databases, search terms, number of publications retrieved and subsequent filtering process results.

	Database	Search Terms	Results
Initial searches	CINAHL	Wound* AND Amnion* OR Chorion* (limiters: Full text; Abstract available; English)	135
	Cochrane	Wound* AND Amnion* OR Chorion*	49
	Medline	Wound* AND (Amnion* OR Chorion*)	54
	Pubmed	Wound* AND (Amnion* OR Chorion*) (limiters: Full text; 1990-2018; English) (Amnion* OR Chorion*) AND Allograft (limiters: Full text; 1990-2018; English)	475 160
Initial Total		Total publications from Initial searches	873
		Removed duplicates (107)	766
1st Pass		Include: Only (Amnion* OR Chorion*) AND Allograft	332
2nd Pass		Included: Dehydrated / Specifically Excluded: Micronised	35
		Total for review	35

Basic properties of the dehydrated human amnion/chorion membrane (dHACM) product EpiFix

A series of publications produced by Koob and colleagues examined the *in vitro* and *in vivo* characteristics and properties of the most widely used dHACM known as EpiFix (MiMedx, Marietta, GA, USA) in a variety of cell types including dermal fibroblasts (Koob *et al.* 2013, Koob *et al.* 2014a); microvascular endothelial cells (MVECs) and human umbilical vein endothelial cells (HUVECs) (Koob *et al.* 2014b); human bone marrow derived mesenchymal stem cells (BM-MSCs) (Koob *et al.* 2013,

Massee *et al.* 2016a); adipose derived stem cells (Massee *et al.* 2016a, Massee *et al.* 2016b) and haematopoietic stems cells (Massee *et al.* 2016a). These studies all utilised quite similar experimental approaches and techniques to measure: cell proliferation; migration; protein expression and or gene expression; and mouse models for in vivo responses to whole dHACM or extract. Overall, the studies showed that dHACM extract contained a range of GFs and cytokines that were able to induce cellular proliferation and migration to varying degrees, in the cell types tested, above negative control levels but generally not above the positive controls, which usually contained standard growth media including 10% foetal bovine serum (FBS) (Koob *et al.* 2013, Koob *et al.* 2014a, Koob *et al.* 2014b, Massee *et al.* 2016a, Massee *et al.* 2016b). It is also clear from these studies that the dHACM is able to modulate, clinically relevant, gene and protein expression by the cells investigated. Exactly whether or not these same genes and proteins are modulated in human patients is not clear, however, select *in vivo* studies were conducted showed the ability of dHACM to recruit specific cell types and modulate specific functional responses such as recruitment of MSCs (Koob *et al.* 2013) and induction of neovascularisation (Koob *et al.* 2014b). The authors of these studies all declare a conflict of interest as most are employees of MiMedx Group, the manufacturer of the EpiFix dHACM. It is also important to note that the developer of EpiFix has also now developed Revita as an alternative dHACM with similar properties to EpiFix but with additional features which offer some additional advantages for clinicians and patients.

Diabetes related foot ulcers

In a prospective, stratified, randomised, comparative, parallel group, non-blinded clinical trial of the effect of bi-weekly applications of the dHACM EpiFix on diabetic foot ulcer (DFU) healing, 77% (10/13) of patients that received EpiFix healed by four weeks compared to 0% of patients receiving standard care (SC) alone. By six weeks 12/13 patients receiving EpiFix had healed whereas only one of 12 subjects receiving SC had healed. While four patients in the SC group experienced adverse events including development of cellulitis (2); gastrointestinal bleed (1); and pyelonephritis (1), a single patient in the EpiFix group developed pneumonia, respiratory stress and acute renal failure. The authors concluded that the latter was not likely due to the use of the EpiFix allograft (Zelen *et al.* 2013). In a follow up study the lead author, a clinician with extensive DFU expertise, treated 11 patients from the SC group that had not healed within the six weeks of the study described above with bi-weekly application of EpiFix. During the previous study these patients collectively exhibited a mean increase in wound size whereas treatment with EpiFix resulted in a consistent decrease in wound size such that by week two 82% and week four 100% of the patients had exhibited a 50% decrease in wound area. All but one patient completely healed within 12 weeks of treatment with an average of approximately 4.23 ± 3.1 weeks to complete healing. Almost half (45%, 5 patients) the patients healed completely with a single application of EpiFix, one with two applications, one with three applications, two with four applications and one with five applications (Zelen 2013).

While in both of the above studies the authors acknowledge that they did not compare the effect of EpiFix with other advanced care products (Zelen 2013, Zelen *et al.* 2013). Thus in a subsequent study to examine the effect of weekly applications of dHCAM the authors also compared the effect of a bioengineered skin substitute, Apligraf® (Organogenesis, Canton, MA), on diabetic wound healing. Apligraf is a class III medical device which is a bi-layered allogeneic cultured skin substitute derived from male neonatal foreskin tissue. The graft matrix is derived from bovine type I collagen containing human foreskin fibroblasts and a keratinised epidermal layer. Apligraf has a shelf life of approximately 15 days and is supplied sealed in a polyethylene bag containing nutrient medium and a 10% CO^2 atmosphere. Whereas, EpiFix, a dHACM with a 5 year shelf life and composed of human collagen based extracellular matrix that contains important biological molecules such as growth factors, is supplied in a sealed sterile bag under ambient conditions. Thus, while EpiFix and Apligraf, while used for similar purposes are quite different in terms of their production and ready-for-use composition. Patients who

met rigorous inclusion and exclusion criteria and exhibited a less than 20% reduction in wound size following a 2 week run-in period, during which they received conservative wound care, were randomly assigned to receive either Apligraf, EpiFix or SC (n = 20 per group). While there was no statistically significant differences in patient demographics or wound characteristics between the groups the authors found that after 4 weeks of the trial 35%, 85% and 30% of patients receiving Apligraf, EpiFix and SC respectively, had achieved complete healing. The EpiFix treatment had significantly increased healing rates over Apligraf and SC (p = 0.001). After 6 weeks of treatment 95% of patients that received EpiFix exhibited complete healing compared to only 45% (p = 0.006) and 35% (p = 0.001) of patients that received Apligraf and SC respectively. A cost analysis indicated that the EpiFix cost 81.9% less for the graft material compared to Apligraf and averaging at $1669 USD and $9216 USD per patient respectively. With respect to safety, a single patient that received EpiFix developed cellulitis and infection in the wound 1 week after the beginning of the trial and was subsequently treated with sharp debridement, antibiotics and silver dressing. As such the patient had to withdraw from the study, interestingly however, the patient had exhibited a 41.7% reduction in their wound size at the time of withdrawal. Given the other 19 patients in the EpiFix group did not experience any adverse events the authors concluded that this was not likely related to EpiFix (Zelen *et al.* 2015). Similarly, the other 4 reported adverse events that occurred in patients that received Apligraf and SC were determined to be unrelated to the use of Apligraf or SC during the study.

In a similar study of 100 patients that received either Apligraf (n = 33), EpiFix (n = 32), or SC (n = 35) after randomisation and withdrawals. The groups were well matched in terms of demographics and wound characteristics. Over the 12 week study period complete healing occurred in 73%, 97% and 51% of patients receiving Apligraf, EpiFix and SC respectively (p = 00019). Mean days to healing were 47.9, 23.6 and 57.4 days for Apligraf, EpiFix and SC respectively ($p = 3.2x10^{-7}$). A cost comparison similar to that in the earlier study indicated that the EpiFix group received 58% fewer grafts and 94% less graft material and an 83% lower Median cost than Apligraf. Four subjects' receiving Apligraf and thirteen subjects receiving SC withdrew from the study after 6 weeks when their wounds did not exhibit a reduction of more than 50% in size. An additional subject withdrew at 10 weeks after their wound increased in size and five patients that received SC discontinued the intervention between 3, 4, 5 and 11 weeks. In all, ten adverse events were recorded of which: seven included wound or foot infections; one urinary tract infection; and one car accident. None of the adverse events were attributed to the use of EpiFix or Apligraf. Overall, the dehydrated amniotic membrane product EpiFix exhibited significantly superior healing of lower extremity wounds in patients with diabetes and cost effectiveness compared to Apligraf and SC (Zelen *et al.* 2016). Indeed, wounds that received the dHACM were almost 6 times more likely to heal compared to wounds that received SC and there was no significant difference between the effect of Apligraf and SC on wound healing. Moreover, the authors make the point that the U.S. Food and Drug Administration (FDA) accepted a hazards ratio (HR) of 1.59 (95% CI 1.26 - 2.00) for the approval for use of bioengineered skin substitutes such as Apligraf (Veves *et al.* 2001) whereas the HR determined from their current study was 5.66 (adjusted p 1.3×10^{-7}). However, the authors acknowledged that the more advanced standard-of-care of their study may have contributed to the improved healing rates.

While much of the above mentioned work has been carried out by the same core authorship group, a separate team of researchers conducted a very large retrospective analysis of diabetic foot ulcer healing data from the WoundExpert electronic medical record (EMR) database. The EMR contained data from 3000 wound care facilities across the USA and employed a patient screening regime as close as possible to the inclusion and exclusion criteria employed in two studies by Zelen and colleagues (Zelen *et al.* 2013, Zelen *et al.* 2015). Similar to the Zelen *et al* RCTs the retrospective study performed by Kirsner and colleagues aimed to compare healing of DFUs treated with the bioengineered living cellular construct (BLCC) Apligraf (as described above) and the dHACM EpiFix. Whereas the Zelen *et al* studies were performed over a 12 week study period, the retrospective study performed by Kirsner *et al*., 2015 included data from a 24 week period. Data from 248 centres was collated resulting in 994

wounds that had received the Apligraf and 464 that had received the dHACM. Wounds: not on the feet; < 1 cm^2 or ≥ 25 cm^2; with a duration of > 52 weeks; received alternate skin substitute within 28 days prior to treatment; > 20% healing within 14 days of treatment; without follow-up wound area measurement were excluded from the study. This resulted in a comparison between 163 wounds from 155 patients that received Apligraf and 63 wounds from 63 patients that received the dHACM treatment, collectively from 99 centres across the USA. The results contradicted those of the Zelen *et al* RCTs in that the authors reported that wounds treated with Apligraf healed at roughly twice the rate compared to wounds treated with EpiFix. The authors directly question the results of the Zelen RCTs and suggest that the stark difference may be a result of the Zelen RCT's: relatively small sample size; the tight patient selection criteria used; and geographical proximity of the trial centres. The authors point out that the median wound size was bigger and the number of allograft applications was different in their retrospective study. They also pointed out that clinicians may have a patient selection and treatment pattern bias for the use of either Apligraf or EpiFix. Finally, the authors also report fewer required average number of application of Apligraf per wound and greater cost savings. While there is no suggestion of overt or intentional bias or impropriety in the reporting of the study findings, it is important to note that four of the five authors either consult for or are employed by Organogenesis Inc, the manufacturer of Apligraf (Kirsner *et al.* 2015). Similarly, four of the six authors of Zelen *et al*., 2015 and all of the authors in Zelen *et al*., 2013 either consulted for or received research funding from MiMedx Group Inc, including for the reported studies. The difference between the studies could best be described as distinguishing efficacy (RCTs) versus clinical effectiveness in a broader setting via the observational studies.

Interestingly, Smiell *et al.,* 2015 conducted a large observational study of 165 patients with 179 chronic wounds of different aetiologies and a range of co-morbidities that were treated with the decellularised, dehydrated human amniotic membrane (DDHAM) Biovance® (Alliqua Biomedical, Langhorne, PA, USA). The study did not include randomisation, blinding or a control group and the authors rationalise this approach by arguing that it provides for a more "real world" or "realistic test of effect of an intervention for patients". The stated aim of the study was to obtain "evidence of clinical benefit" of application of DDHAM allograft and to examine which wound types most benefit from such application. While the opinion of this author (TP) is that this rationale has significant flaws the study did seem to serve as an analysis of potential adverse events in a clinical diverse population. While the authors found that there was perhaps a positive effect of using DDHAM with an estimated potential 20% increase in wound closure over other published RCTs for various wound types, the range of comorbidities make this type of effectiveness assessment somewhat unreliable. The reason for inclusion here is that the authors did not report any adverse events attributed to the use of the product across the diversity of clinical conditions represented in the study population (Smiell *et al.* 2015).

Similarly, a small study of five patients with underlying type 2 diabetes or associated co-morbidities and wounds of the lower extremity that had initially exhibited recalcitrance against conservative treatment, showed 100% healing across a 11.5 week period (average 7.3 wks) following treatment with dHACM (Penny *et al.* 2015). The authors concluded that the dHACM was effective in helping to induce healing and that healing times might have been more rapid with improved patient compliance with regard to maintenance and management of dressing integrity. They also suggested that the healing rates were comparable to those previously reported in in the RCT described above by Zelen et al., 2013 (Zelen *et al.* 2013, Penny *et al.* 2015).

Again a similar case study series of five patients with various wound types that had persisted for longer than 30 days without healing following standard care, were non-randomly recruited and offered single application of EpiFix dHACM (Mrugala *et al.* 2016). One wound did not exhibit a healing response following application of dHACM and on further investigation it was found that the patient had an occluded inferior vena cava resulting in extremely high venous tension in the lower limbs and in the wound. Despite this, epithelial island formation was evident in the wound bed at 3 weeks post dHACM

application. Mild compression therapy over the following 6 months resulted in healing of the wound. On average the remaining four wounds exhibited a 72% reduction in wound area in the 3 weeks following application of dHACM, with complete healing in an average of around 9 weeks. The authors report that the handling features of the dHACM were superior to other cryopreserved or bioengineered advanced skin replacement products and concluded that the dHACM generally promoted healing despite the varied co-morbidities of each patient and the varying wounds. The authors also discuss the literature related to the varied use of dHACM and the reader of this review is directed to the Mrugala *et al.*, 2016 paper for additional summary of the literature (Mrugala *et al.* 2016).

Another case study of eight patients with DFUs showed similar healing trajectories with the use of a "dehydrated amniotic-derived tissue allograft" (DAMA; Amnioexcel; Derma Sciences, Inc, Princeton, New Jersey), with a mean time to healing of 9.2 weeks +/- 3.67 weeks and an average of 2.11 applications of the DAMA (Rosenblum 2016).

An interesting case study of an individual 77 year old woman who presented with a complicated clinical history including type II diabetes, venous insufficiency, hypertension, hyperlipidemia, macular degeneration and obesity and had undergone surgery for triple coronary bypass, hip replacement, internal fixation of femur fracture, shoulder surgery and sternal infection (Snyder *et al.* 2015). The patient was on a complex pharmaceutical regimen and presented with three small but painful lesions on her right anterior shin of four months duration. The initial treating clinician suspected an underlying venous aetiology and treated the patient with debridement and multilayer compression therapy, however the wounds increased in size coalesced and became extremely painful (10/10). The patient ultimately sought a second opinion by attending the author's clinic. After extensive unsuccessful conservative wound management approaches and a rule-out diagnostic approach the wound was diagnosed as pyoderma gangrenosum (PG). The multi-disciplinary team treated the wound accordingly for three months with little to any improvement before application of dHACM. The wound was 103 cm^2 prior to initial dHACM treatment and at the 1 week follow-up the patient reported a reduction in pain to 5/10 within hours and 0/10 within days of the dHACM application. The wound had reduced by 27% and to 57.96 cm^2 (56% reduction) over 3 weeks with 3 applications of the dHACM. After 7 months the wound had still not healed but the patient was without significant pain and no adverse effects were observed related to the dHACM. The authors recommended more extensive trials to determine the efficacy of dHACM for the treatment of PG. The lead author declared a conflict of interest as a consultant for MiMedx Group (Snyder *et al.* 2015).

Venous leg ulcers

A recent registered, 16 week, randomised, controlled, multicentre clinical trial (NCT02011503) to examine the efficacy of dHACM treatment on non-healing full thickness venous leg ulcers (VLUs) was conducted using 109 participants randomly assigned to groups that received multilayer compression therapy (MLCT) with or without application of dHACM allograft (Bianchi *et al.* 2018). Participants were assigned to the groups following recruitment according to rigorous inclusion and exclusion criteria and a standardised run-in screening period during which they received 2 layer compression therapy. Patients whose wounds were between 1 and 25 cm^2 following debridement and had not decreased by more than 25% during the 2 week run-in period were assigned to the treatment groups and entered the clinical trial period. The authors found that a the 12 week primary endpoint period, 60% of VLUs treated with the dHACM exhibited complete healing compared to 35% of those treated with MLCT alone ($p = 0.0128$). By the 16 week follow-up time point the percentage of participants that exhibited complete healing of their VLUs was 71% and 44% for those with or without receiving dHACM allograft respectively ($p = 0.00625$). The average reduction in wound area at 12 weeks was 66% for dHACM treated wounds compared to 40% for those that received MLCT only. At 16 weeks the average

reduction in wound area was 72% and 39% respectively. These data indicated that on average wounds that had not healed by 12 weeks of MLCT did not continue to heal. Using a Cox regression analysis of the influence of potential co-variates the authors found that that the most influential factor on healing was the application of the dHACM. Indeed, wounds were more than twice as likely to heal within 12 weeks is treated with the dHACM. Interestingly, a relatively large number of adverse events were reported (*35* and *30* with and without dHACM respectively). These were reviewed by the primary investigators and a Clinical Events Committee who concluded none of the adverse events were related to the dHACM or any procedure utilised in the study (Bianchi *et al.* 2018). In their discussions the authors compare their results with the 12 week healing rates of other trials of advanced wound care products, including Apligraf and Dermagraft on VLU healing. The healing at 12 weeks for these other products ranged from 31% healed to 45% healed compared to 60% healed for the dHACM.

The findings from the above study are supported by previous findings of Serena and colleagues who conducted the only other and first registered multicentre, randomised, controlled trial (NCT01552447) on the efficacy of dHACM (EpiFix) on VLUs (Serena *et al.* 2014). Participants that met substantial inclusion and exclusion criteria were entered into a screening period during which participants were treated with MLCT prior to entering the study if they continued to meet the inclusion and exclusion criteria. The participants (n=84) were randomly assigned to three groups, those that received either one or two applications of the dHACM in addition to MLCT or MLCT alone. Significantly more participants (62%) who received dHACM, exhibited >40% reduction in wound size over the 4 week study period, the primary outcome, compared to those who received MLCT alone (32%) (p= 0.005). These findings were challenged in a letter to the editor by Dickerson and Slade, 2015, who suggested that since 30% to 70% of VLUs will heal with MLTC, a "run-in period" should have been employed, during which wounds would have been treated with MLTC. According to the authors, only wounds that did not respond to this period of care should have been enrolled and randomised in the trial (Dickerson and Slade 2015). Furthermore, the letter also criticises the use of the 4 week endpoint, suggesting that other literature found that this endpoint was only correct about 70% of the time (Dickerson and Slade 2015). The Serena *et al*., 2014 study, however, did include a "screening period" of 2 weeks of MLTC after which wounds that continued to meet the inclusion criteria were admitted to the study and randomised to the 3 treatment groups detailed above. It is possible that wounds that had responded to the MLTC during the screening period, but were still greater than 2 cm^2 in area were enrolled into the study. This could have skewed the results, since two of the three treatment groups included application of dHACM in addition to MLCT, thus a greater number of actively healing wounds would likely have been randomly assigned to a group receiving dHACM (Dickerson and Slade 2015). A follow-up retrospective investigation of the Serena *et al*., 2014 study outcomes went some way toward addressing the concerns raised by Dickerson and Slade regarding the 4 week end point. The follow-up investigation indicated that the use of healing status at 4 weeks of treatment is a reasonable surrogate for healing status at 24 weeks with 72% of participants exhibiting the same healing status at 24 weeks as they exhibited at 4 weeks regardless of group (Serena *et al.* 2015). Comparisons between wounds treated with one and two applications of dHACM and MLCT alone were secondary outcomes. There was no significant difference in the proportion of wound reduction between participants that received either one or two applications of the dHACM, indicating that a single application was enough to induce significantly faster healing than MLCT alone. From the dHACM groups, seven patients reported nine adverse events which included, falls, worsening COPD, syncope and cellulitis in the non-study leg. All were determined to not be related to the use of dHACM. The authors make the point that the "results may not be generalised to other amniotic membrane products" due to differences in preservation techniques and membrane configurations that may affect product effectiveness (Serena *et al.* 2014). It is important to note that the proprietary PURION® process (MiMedx Group Inc.) is used to separate the placental tissues including the chorion and amnion washes out the intermediate layer and re-laminates the chorion and amnion prior to dehydration and terminal sterilisation which produces the dHACM EpiFix. Whereas, the Clearify™ process for the production of Revita® involves a proprietary cleaning process in which the amnion,

chorion and intermediate layer are not separated prior to dehydration and terminal sterilisation thus resulting in a product that has undergone less manipulation than the EpiFix product (Stimlabs 2018).

An observational study, which included 8 participants with ulcerated free flaps and venous insufficiency and/or lymphedema, was conducted to evaluate the use of dHACM in addition to conservative treatment until healing (Miranda and Friedman 2016). The authors found that 3 of the 4 recipients of dHACM exhibited complete healing within 10 – 66 days (mean 33 days) compared to healing in 41-195 days (mean 87 days) for recipients who received conservative treatment only. The fourth patient to receive dHACM repeatedly removed the graft and was removed from the study after receiving four applications of the graft. Moreover, unlike the other recipients the graft was located on the proximal lower leg and therefore was not able to receive effective MLCT at that site. Otherwise the grafts were well tolerated and the authors conclude that while the study cannot be generalised they demonstrated the feasibility of using dHACM for the treatment of ulcerated free flaps. (Miranda and Friedman 2016).

Burn Wounds

In February 2017 a supplementary issue of the clinical journal Annals of Plastic Surgery, that focussed on the utilisation of dHACM for the treatment of burn wounds. While the issue only examined the use of dHACM from the MiMedx group. As such a majority of authors declared conflicts of interest (COI) on four of the six articles. The six papers were largely reviews of how amniotic tissue in its various formats has been utilised in different burn contexts. There were two informative observational case study based papers included in the issue, detailing the use of dHACM in various burn contexts. While RTC studies would be required to enable formulation of conclusions with regard to efficacy, such studies are fraught with issues related to the ethics of randomisation of treatment where the outcome could impact long term scar outcome with its associated requirement for revision surgeries, especially in children. While not utilising dHACM, one early RCT examined the comparative effect of using topical antimicrobials with or without cryopreserved amnion in 102 paediatric patients (n=49 and 53 respectively) with partial thickness burns to the face, head and/or neck (Branski *et al.* 2008). The authors found that the use of the amnion resulted in significantly fewer dressing changes and the healing time was slightly but not statistically significantly faster and there was no difference in the scarring outcome. While not able to be objectively measured the authors made the subjective observation that the amnion group tolerated the fewer dressing changes better than the antimicrobial group only suggesting that there may have been a benefit in terms of pain reduction in these patients. Few other large scale RCTs have been performed using dHACM in burns.

The case studies and reviews in the above mentioned supplementary issue suggest that dHACM is safe and may reduce inflammation, limit itch, pain and perhaps scarring (Glat 2017, Glat and Davenport 2017, Reilly *et al.* 2017, Tenenhaus 2017). Additional RCTs or appropriate pre-clinical model trials should be conducted to better answer some of these issues. Appropriate study design would be of critical importance. Animal models of burns are limited especially with regard to hypertrophic scarring and are generally restricted to pig studies as there are differences in skin structure and healing processes between humans and rodents. Moreover, the ability to design studies in such models that are able to measure pain and itch would be difficult.

The case studies reported in the above issue and others serve to demonstrate safety, general utility and the extent of any adverse outcomes. While it might be reasonable to be cautious of the lack of reporting of adverse outcomes in instances where clear COIs occur, the review articles citing previous studies which showed no adverse events related to the use of dHACM or amniotic membrane products is reassuring in this regard (Glat 2017, Glat and Davenport 2017, Reilly *et al.* 2017, Tenenhaus 2017).

Other applications

Subach and Copay (2015) utilised the dehydrated human amnion/chorion membrane (dHACM) product AmnioFix (MiMedx, Marietta, GA, USA) in a clinical trial of five male patients undergoing transforaminal lumbar interbody fusion (TILF) with posterior instrumentation. While the study numbers were small they authors reported limited dural adhesions and fibrosis within the epidural space. The authors self-report that the dHACM resulted in lower levels of fibrosis and dural adhesions compared to other products they had used previously and no spinal fluid leakage (Subach and Copay 2015).

AmnioFix (MiMedx, Marietta, GA, USA) was also used in 58 patients who underwent nerve sparing robot assisted laparoscopic prostatectomy (NS RARP) to determine its functional benefit in reducing the impact of physical traction injury of the neurovascular bundle (NVB) during surgery. Patient return to continence and potency was evaluated using validated questionnaires against a control group who did not receive grafts. Return to continence and potency were both achieved significantly sooner (1.21 mo vs 1.83 mo, $p = 0.033$; and 1.34 mo vs 3.39 mo, $p = 0.007$ respectively). There were no adverse events in either group. The authors attribute the result to the range of neurotrophic growth factors present in the allograft material which they suggest may lead to more rapid repair of nerve injury. However, authors also recognised the control cohort data was retrospective and therefore there was no prospective randomisation of patients to the trial. Importantly, the authors reported no adverse effects (Patel *et al.* 2015). In a follow-up retrospective study of prospectively collected data the same authorship team examined a large cohort of patients (n=940) who underwent RARP, a subset of whom (n=235) received a dHACM circumferential wrap around their NVB with the remaining (n=705) receiving RARP alone (Ogaya-Pinies *et al.* 2017). The primary outcome of this study was to examine the resolution of potency following surgery with or without dHACM. The authors report significantly higher rate of return to potency at 1, 3, 6 and 9 months but not 12 months post-surgery in the cohort that received dHACM ($p < 0.001$; $p < 0.028$; $p < 0.007$; $p < 0.044$; $p < 0.463$ respectively) regardless of the degree of nerve sparing employed. Overall, the greatest benefit of dHACM treatment was in the sub-cohort that were <50 years old ($p < 0.0056$). There was no difference between the dHACM cohort and the RARP only cohort (1.70% and 1.99% respectively; $p < 0.544$) with respect to the return of serum prostate specific antigen (PCA) levels (biochemical return (BCR)) > 0.2 ng / mL, indicating that the repair promoting dHACM did not exhibit oncogenic activity. The authors acknowledged that the study lacked prospective randomisation element and that the patients knew that they were receiving the dHACM allograft and therefore may have an introduced a placebo bias to the study. Overall however, the study appeared to indicate that the use of dHACM is safe and may have efficacy in promoting the return of potency following RARP especially to those below 50 years of age (Ogaya-Pinies *et al.* 2017).

AmnioFix was also used in the first robotic vesicovaginal fistula repair in a 66 year old women who had developed the fistula as a result of prior radiotherapy and radical hysterectomy related to locally advanced cervical cancer, and had resulted in total uncontrolled urinary incontinence for over 6 months (Price and Price 2016). In this instance the dHACM was sutured in place and the authors reported that there were no intraoperative or post-operative complications and at 5 months post operation the fistula had not recurred and the incontinence had resolved. The authors concluded that the use of the dHACM was successful but commented on the fragility of the membrane AmnioFix membrane (Price and Price 2016). As a point of difference the proposed Revita® product is more robust than the AmnioFix product since the placental tissue is not delaminated during the processing of the Revita® product (Stimlabs 2018).

Recently EpiFix was used in the endoscopic repair of a gastric leak in a 43 yr old male patient 6 weeks following gastric sleeve surgery. At a follow up endoscopy the leak site had healed well indicating the site had healed well (Sousa *et al.* 2017).

A dHACM product, AlphaPatch from Amniotic Therapies (Dallas, TX, USA) was applied to a non-healing surgical wound on the right anterior knee, a result of vasculopathic venostasis following total knee arthroplasty (Riordan *et al.* 2015). The allograft was applied 43 days after the initial knee replacement and had developed a central scab by follow up 2 weeks later. The healing progressed through development of a full scab by 4weeks, release of the scab and complete reepithelialisation at 8 weeks to complete healing with full range of motion of the knee at 10 weeks. The authors indicate that while further studies are required they assert that the utilisation of dHACM is a viable adjunct treatment to existing wound care management (Riordan *et al.* 2015). The first / corresponding author and senior author declare a conflict of interest in that they are shareholders of Amniotic Therapies, the manufacturer of AlphaPatch.

Amniotic allograft has been used extensively in ophthalmology. An interesting recent case study indicated safe use of dHACM (Ambio5; IOP Ophthalmics, Costa Mesa, CA) for the repair of a wound caused by and extruded scleral buckle which is a prosthetic used for retinal detachment repair. The large defect to the conjunctiva was successfully repaired with the use of the dHACM following an unsuccessful period of conservative care (Grewal and Mahmoud 2016).

EpiFix was used to treat wounds that resulted from Mohs surgery to remove basal cell carcinomas on the lower eyelid in a small case series (Wisco 2016). These wounds can undermines the structural integrity of the eyelid and these patients are therefore at risk of suffering from ectropion (turning out of the lower eyelid). The two female patients refused surgical intervention to repair their wounds and so were offered dHACM as an alternative treatment. While the male patient had a complication during surgery associated with excessive bleeding due to medications he was taking for other health conditions and therefore dHACM was used instead of healing by secondary intention due to the increased risk of ectropion formation. All patients healed without complication, scarring or ectropion formation in 45 days, 15 days and 18 days for the 2 females and male respectively. All of the patients reported that they experienced little pain and satisfaction with the results of their treatment (Wisco 2016).

Summary

In summary with regard to the use of dHACM (namely EpiFix and also Revita based on currently available data) for the treatment of chronic non-healing wounds it is currently unclear to exactly what extent that the product accelerates wound healing, although it appears to improve healing rates over and above standard care. It is likely that this is due to the inherent properties of the graft to initiate a healing response in the wound tissue rather than driving the healing response to resolution. Support for this hypothesis would appear in the need in some cases for additional applications of dHACM product. This obviously has occurred in the studies described above on a case by case basis. Critically, it appears that the dHACM products investigated, as manufactured and presumably applied in accordance with the manufacturer's instructions, is safe and does not cause adverse reactions in patients. The efficacy for the use of dHACM products including Revita in specific wound healing and surgical applications will be better determined through specific well designed future RCTs. While not specifically reviewed herein, how these dHACMs elicit their biological effects will be better understood through additional intelligently executed mechanistic studies in both *in vitro* and *in vivo* systems.

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Appendix P

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