Wound granulation tissue with an extracellular matrix graft in chronic limb-threatening ischaemia: a histopathological examination

Key words:
- *Debridement*
- *Extracellular matrix graft*
- *Granulation tissue*
- *Histopathology*
- *Wound infection*

Background: An extracellular matrix graft (OASIS wound matrix, OASIS) reportedly provides an improved environment for chronic wound healing. This study aimed to evaluate the effectiveness of the extracellular matrix graft for chronic limb-threatening ischaemia (CLTI) in histopathological examination. **Methods**: The extracellular matrix graft was applied to half of the wound postsurgery; the other half served as the control. The granulation tissues were biopsied after one week and on the reconstruction day. The cells were counted during tissue examination. **Results**: Seven patients achieved complete healing. In 1 week, the extracellular matrix graft group had more cells than the control group (p<0.05). **Conclusions**: The extracellular matrix graft improved wound healing in CLTI.

***Yuta Terabe, Nobuhito Kaneko, Shinya Hata, Keisuke Nakabayashi, Akihiro Matsui, Hiroshi Ando** all at* Limb Salvage center, Kasukabe Chuo General Hospital

Chronic leg and foot ulcers, such as diabetic foot ulcers, venous leg ulcer, and arterial leg ulcer, have been regarded as a global clinical problem. Chronic limb-threatening ischemia (CLTI) is one of the major causes of delayed healing in lower limb ulceration (Conte et al, 2019). The prevalence of CLTI is increasing and is estimated to reach 2.8 million in 2020 (Diaz-Sandoval, 2019).

As in this study, many patients with CLTI are older. Therefore, the three main joints are often already deformed (of course, if they can walk, they are often mild deformity). In addition, since it is necessary to off-loading the affected side, the burden increases due to changes in gait, and the use of off-loading device causes a difference in leg length. These often complain of three main joints and lower back pain during treatment. There are no cases requiring treatment for joint deformity during the treatment period, and there are no papers describing these, but patients' complaints in clinical practice are often heard.

The aim of CLTI treatment is not only limb salvage but also gait salvage. Minimising foot amputation is important to acquiring the gait salvage. Amputating the foot provide the unbalance of bilateral leg and increasing load of the amputation side. Therefore, over-loading of the lower leg joint (ankle joint and several foot joints) give upper leg joint (knee joint and hip joint) the burden. Long-term CLTI treatment was sometimes required to leave the foot as much as possible. However, long-term CLTI treatment can cause disuse syndrome. Disuse syndrome at the time the ulcer heals reduces the patient's quality of life. Hence, patients with CLTI need to be healed and to resume their usual life as soon as possible. OASIS extracellular matrix (ECM) (OASIS) has improved the healing of chronic ulcers compared with the standard of care (Mostow et al, 2005; Brown-Etris, 2019). In Japan, OASIS has been used clinically since 2015. It consists primarily of a collagen-based ECM from swine small-intestine mucosa. The ECM of OASIS contains glycosaminoglycans (Hodde et al, 2007), proteoglycans, fibronectin (McPherson and Badylak, 1998) and growth factors such as basic fibroblast growth factor (Hodde and Hiles, 2001) and transforming growth factor-β (TGF-β).

CLTI treatment needs revascularization, off-loading, and infection control (Conte et al, 2019). Wounds that heal by improving only ischaemia are a part of CLTI. The treatment methods of almost CLTI are mixed therapy and multidisciplinary approach.

Revascularization options include endovascular treatment (EVT) and peripheral

Table 1. Patients characteristics		
		Patients
Patient number (n)		7
Age (years)		73.1±13.9
Diabetic mellitus (n)		6
Chronic kidney disease (5Gd) (n)		1
Wound healing rate (%)		100
Wound healing term (days)		21.4±11.0
Coronary artery disease (n)		3
Cerebrovascular disease (n)		0
Skin perfusion pressure (mmHg)	dorsal	25.8±6.31
	plantar	28.1±9.67
Ulcer size post-debridement (cm^3)		24.8±10.6 (cm^3)
Target vessel region (n)	above the knee	2
	below the knee	4
	below the ankle	1
Ulcer area (n)	toe	2
	dorsal	4
	plantar	1
Debridement area (n)	dorsal skin	4
	toe amputation	2
	trans metatarsal amputation	1

**Number of age and wound healing: average± standard division*

bypass surgery. Most revascularizations are undertaken as EVT in the world. EVT is the target of several below-the-knee cases. However, restenosis is its severe complication. Almost below-the-knee cases return the pre-EVT vessels for 3 months (Iida O et al, 2012). Therefore, CLTI needs to heal within 3 months after revascularization. OASIS has the potential to promote CLTI treatment by ensuring that the resulting wound heals quickly.

In this study, we aimed to evaluate the efficacy of the OASIS in promoting wound healing in CLTI cases. We presented a side-by-side test to assess the effectiveness of the OASIS in CLTI treatment

Method

Patients

The study protocol and informed consent statements were reviewed and approved by the ethics committee of Kasukabe Chuo General Hospital (ethical number 1710-1). This was a prospective study in patients with CLTI (all patients had forefoot ulcer), who were diagnosed according to the clinical presentation, and recruited consecutively, on presentation. They agreed to the terms of the trial and signed the informed consent form.

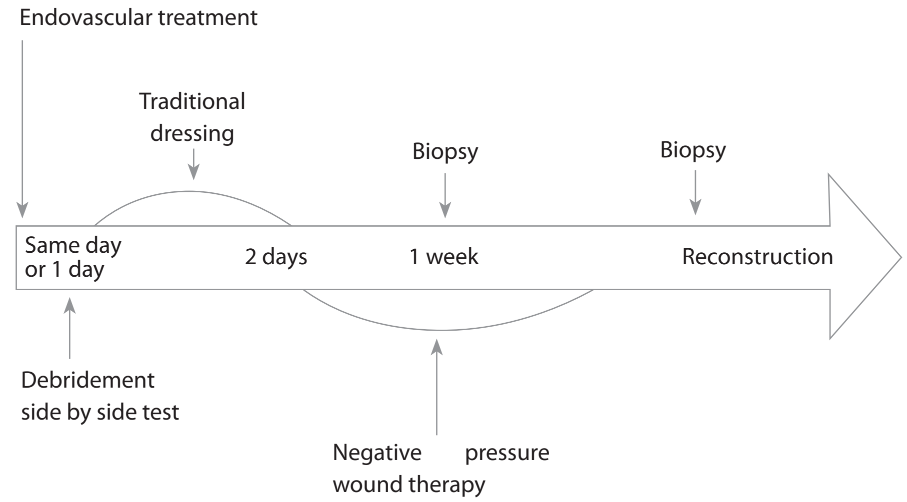
We included patients who were over 18 years old and who exhibited an ischaemic foot ulcer assessed by skin perfusion pressure (SPP) or leg artery ultrasound examination (*Table 1*). The abnormal SPP value was less than 40 mmHg. An abnormal leg artery ultrasound examination was described as having stenosis and/or occlusion of at least one below-the-knee vessel. In addition, their ulcers were over 9 cm^2 (length × width) in size.

Study design

This prospective, side-by-side test conducted at one institution (Kasukabe Chuo General hospital, limb salvage center) enrolled patients who underwent vascular examination (SPP and/or leg artery ultrasound examination). They also had undergone EVT. The EVT cases underwent debris or dead tissue removal (debridement). Measurements were taken post debridement to establish the full extent of the wound.

Conversely, the exclusion criteria were as follows: no arterial disease (SPP >40mmHg, and/or the leg artery ultrasound examination detected normal vessels); allergic to porcine products; or religious or cultural objections to porcine product use.

Figure 1. Treatment process



Ulcer treatment

After the EVT, the patients underwent surgery (debridement and amputation), removing a part of the foot. The extracellular matrix graft (OASIS, Smith & Nephew) was applied to the resection stump after the surgery, it was placed upon a half portion of the wound bed and moistened with sterile saline. The other half-side served as the control area. The extracellular matrix graft area was placed on the more severe wound bed (more distal area or more inflammation area in wound bed) than control area (given the extracellular matrix grafts ability to promote the wound healing, we placed extracellular matrix grafts on the more severe side). Subsequently, secondary dressings, silicone-faced mesh-type wound dressing (SI Aid Mesh, ALCARE Co., Ltd.), gauze, and a bandage were applied to protect the healing environment and to maintain the direct contact of extracellular matrix graft with the wound bed. The extracellular matrix graft is only applied once and after two days the dressings, except for the silicone-faced mesh-type wound dressing were removed, and the wounds were assessed for any presence of infection and bleeding. Without wound complication, topical treatments including negative pressure wound therapy (NPWT) were used across the whole wound.

Protocol and evaluations

Following treatment initiation after EVT and first operation, we evaluated the patients weekly until to the time of wound closure. At baseline and each follow-up visit, we evaluated and recorded the ulcer status by taking photographs and wound measurements. Wound length was defined as the longest edge-to-edge measurement of the ulcer, wound width was measured at the widest point perpendicular to the length, whereas depth was the deepest vertical measurement using a cotton swab.

Furthermore, patients received standard of care treatments, which included wound cleansing, maintenance debridement as necessary, and dressing changing. All patients underwent negative pressure wound therapy (V.A.C.® therapy: KCI) without bleeding and necrotic tissue occurrences of the wound bed.

Collection of the wound granulation

Wound tissues were collected one week after the surgery and the reconstruction day when the wound bed was covered by granulation and then biopsied by punch biopsy (φ3 mm). The center of each wound bed, extracelular matrix graft and control areas, were extracted. The punch biopsy instrument was held perpendicular to the lesion surface. The instrument was pressed down into the lesion until the root of the instrument blade was buried. Then, a full-thickness granulation tissue was extracted and examined.

Wound healing assessment

To assess wound healing using the wound size, we collected the measuring data at one week postsurgery and on the reconstruction day and then calculated the wound volume (length × width × depth). The reduction rate of each group was compared (postsurgery day versus one week postsurgery, postsurgery day versus reconstruction day). The reduction rate (%): (pre–wound volume – post–wound volume) /

Table 2. Wound reduction response

	One week versus presurgery	Reconstructive versus presurgery
Extracellular matrix graft (%)	–0.2	–42.9
Control (%)	–9.6	–21.8
***p*-value**	0.302	0.072

pre–wound volume × 100.

Pathological assessment

The tissue specimen sections were stained using ordinary hematoxylin and eosin (HE) and then examined using a stereo intravital microscope (SZH 10, Olympus, Japan) at 200× magnification.

Using ramdon sampling we selected three fields in each specimen to further assess wound healing and counted the HE-positive cells in each field. The granulation area of the tissue specimen sections was divided into blocks, and three fields were randomly selected to correspond to the random number table. The cell density of each field was defined as follows: Cell density = cell count/area of the selected fields (number/μm^2). The wound healing ability of each specimen was expressed by the average cell density of the three selected fields.

Statistical analysis

The sample size was the minimum size of this analysis. Study data were collected from the cell densities and the reduction rate and expressed as means and standard deviation. Differences between extracellular matrix graft-applied wounds and traditional-treatment wounds were evaluated using the Student's t-test at a one-tailed $p<0.05$ level of significance. All statistical data were analysed using the R software (Saitama Medical Center, Jichi Medical University, Japan).

Results

Patients

We recruited seven patiets to this study who met the inclusion critrea. The patients characteristics, wound detaols and comorbidities are shown in *Table 1*.

Healing response

All patients were healed. All their wounds were covered with a full-thickness or split-thickness skin graft. No side effects were observed. The average healing time was 21.4 days (range: 7–41 days).

The wound volume in all patients lowered. In one week, the wound reduction in the control area was smaller than that in the extracellular matrix graft-applied area (extracellular matrix

Figure 2. Pathological examination

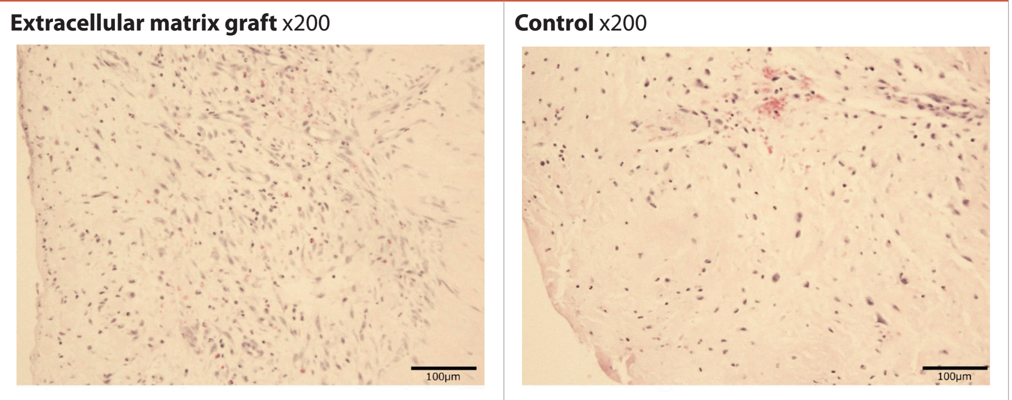


Table 4. Cell density

	Cell density ($n/\mu m^2$)	Standard deviation	*p-value*
Extracellular matrix graft	0.001893096	0.001021846	$p>0.05$
Control	0.00121164	0.000361754	-

graft −0.2% versus control −9.6%; *Table 2*). However, on the reconstruction day, the wound reduction in the extracellular matrix graft-applied area was smaller than the control area (extracellular matrix graft −42.9% versus control −21.8%). Nevertheless, no significant differences were found between the two time periods (one week and presurgery p=0.302, reconstructive and presurgery p=0.072).

Cell density levels following the extracellular matrix graft treatment

The extracellular matrix graft-applied wound area had a higher cell density than the control area, obtaining a significant difference. ($p<0.05$; *Figure 2* and *Table 3*).

Discussion

Some chronic wounds are difficult to heal using traditional therapy. Multiple cytokines (e.g., TGF-β and platelet-derived growth factor) help in healing chronic wounds; otherwise, proinflammatory cytokines and increased MMP levels prevent wound closure. Thus, wound treatment devices are required to increase cytokines and decrease the MMP levels. In line with this, regenerative medicine plays an important role (Hodde et al, 2020).

Regenerative medicine includes stem-cell therapy, which stimulates tissue repair at the damaged site or replaces tissues or organs made or grown *in vitro* (Shi and Ronfard, 2013). Extracellular matrix graft is one of these locally applied regenerative medicines and is a promising solution to chronic wound therapy (Witherel et al, 2016).

The cell density of wounds treated with extracellular matrix graft was confirmed in this study. The study results revealed the potential of extracellular matrix graft for treating chronic wounds, but the details of what the increased cell number means remain unclear. The increase in cells contributes to wound healing, further research is needed to determine which cells are involved and at what time. Hence, increasing the magnification and/or conducting another pathological staining is needed.

The side-by-side test is a useful method of comparing extracellular matrix graft-applied and control areas from a patient's wound. This examination clearly shows the ability of the wound to be healed. However, the examination failed to show the result on wound healing progress, considering that comparing two areas in a single wound by side-by-side test is challenging. Possibly, adjoining areas of the wound are affected in the single wound. Thus, comparing such areas separately is necessary.

Furthermore, we should examine the effect of the mature scar. Thus, more studies are needed to evaluate the progress of wound healing busing the Extracellular matrix graft.

Conclusions and recommendations

Extracellular matrix graft has the ability to promote cells for wound healing. However, long-term observation is required to determine the maturity of a wound healing area. WAS

Conflict of interest: There are no conflicts of interest to declare.

Acknowledgements: The authors would like to thank Enago (www.enago.jp) for the English language review.

References

Brown-Etris M, Milne CT, Hodde JP (2019) An extracellular matrix graft (Oasis ® wound matrix) for treating full-thickness pressure ulcers: A randomized clinical trial. *J Tissue Viability* 28(1):21–6. https://doi.org/10.1016/j.jtv.2018.11.001

Conte MS, Bradbury AW, Kolh P et al (2019) Global vascular guidelines on the management of chronic limb-threatening ischemia. *J Vasc Surg* 9(6S):3S–125S.e40. https://doi.org/10.1016/j.jvs.2019.02.016

Sandoval DL (2019) Critical limb threatening ischemia: the time has come for a multidisciplinary approach. *J Vasc Endovasc Therapy* 4:1

Hodde JP, Janis A, Ernst D et al (2007) Effects of sterilization on an extracellular matrix scaffold: Part I. Composition and matrix architecture. *J Mater Sci Mater Med* 18: 537–43. https://doi.org/10.1007/s10856-007-2300-x

Hodde JP, Hiles MC (2001) Bioactive FGF-2 in sterilized extracellular matrix. *Wounds* 13:195–201

Hodde JP, Hiles MC, Metzger DW (2020) Characterization of the local wound environment following treatment of chronic leg ulcers with SIS wound matrix. *J Tissue Viability* 29(1):42–7. https://doi.org/10.1016/j.jtv.2019.12.003

Iida O, Soga Y, Hirano K, et al (2012) Midterm outcomes and risk stratification after endovascular treatment for patients with critical limb ischaemia due to isolated below-the-knee lesions. *Eur J Vasc Endovasc Surg* 43(3):313–21. https://doi.org/10.1016/j.ejvs.2011.11.025

McPherson TB, Badylak SF (1998) Characterization of fibronectin derived from porcine small intestinal submucosa. *Tissue Eng* 4(1):75–83. https://doi.org/10.1089/ten.1998.4.75

Mostow EN, Haraway GD, Dalsing M et al (2005) Effectiveness of an extracellular matrix graft (OASIS Wound Matrix) in the treatment of chronic leg ulcers: a randomized clinical trial. *J Vasc Surg* 41(5):856–62. https://doi.org/10.1016/j.jvs.2005.01.042

Shi L, Ronfard V (2013) Biochemical and biomechanical characterization of porcine small intestinal submucosa (SIS): a mini review. Int J Burns Trauma 3(4):173–9

Witherel CE, Graney PL, Freytes DO et al (2016) Response of human macrophages to wound matrices in vitro. *Wound Repair Regen* 24(3):514–24. https://doi.org/10.1111/wrr.12423