

# Bio-Engineering tissue and V.A.C. therapy: A new method for the treatment of extensive necrotizing infection in the diabetic foot



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Andrea Armenio\*, Daniela Anna Cutrignelli, Maria Luisa Nardulli\*, Giulio Maggio\*\*,  
Giuseppe Memeo\*\*, Valerio De Santis\*, Giuseppe Giudice\*\*, Cosmo Maurizio Ressa\*

\*Department of Plastic and Reconstructive Surgery, National Cancer Institute "Giovanni Paolo II", Bari, Italy

\*\*Department of Plastic and Reconstructive Surgery, University Hospital Policlinico, Bari, Italy

## Bio-Engineering tissue and V.A.C. therapy: A new method for the treatment of extensive necrotizing infection in the diabetic foot

**AIM:** *The aim of the study is to compare the standard care for progressive necrotizing infection in diabetic foot with a treatment protocol based on the association between autologous fibroblast grafts and vacuum-assisted closure therapy (V.A.C.).*

**MATERIAL OF STUDY:** *A retrospective matched Case-Control study was carried out on 20 patients with diabetic foot infection, 10 treated with the standard care and 10 with our new protocol. Inclusion criteria were: acute diabetic foot necrosis (Wagner III and IV), ulcer size (30 to 80 cm<sup>2</sup>), tendon and bone exposure. Success in the treatment was evaluated as: percentage of healing at the 20th week, time of healing, deambulation, recurrence and major amputation rate.*

**RESULTS:** *A 90% healing rate was observed after 20 weeks in the study group, compared to a 28.6% in the control group. The recurrence rate in the treated areas was 20% in the study group and 100% in the control group. None of the patients in either group required major amputations.*

**DISCUSSION:** *We achieved very promising results by associating autologous fibroblasts grafts and V.A.C. therapy, in comparison with standard care. V.A.C. therapy seems to improve the growth rate of the fibroblasts, probably by sealing the wound and providing a moist environment following the fibroblast graft. The improved neoangiogenesis of the neo-dermis could explain the reduced recurrence rate of the study group.*

**CONCLUSIONS:** *Despite the low number of patients involved and the retrospective nature of the analysis, this study showed a reliable, safe and cost-effective method of treating extensive infection in the diabetic foot.*

**KEY WORDS:** Bio-Engineered Tissue, Diabetic foot, Fibroblast graft, V.A.C. therapy

## Introduction

The worldwide prevalence of diabetes was estimated to be 2.8 percent in 2000 with a projection of 4.4 percent by the year 2030<sup>1</sup>. Foot infections are the most com-

mon problems in people with diabetes (12 to 25 percent)<sup>2</sup>. Neuropathy and ischemia are the principal causes of underlying infections. Involvement of soft tissues and bones (Wagner III and IV) requires extensive surgical debridement and may lead to amputation. Diabetes-induced limb amputations are associated with an increased risk of additional amputation and result in a 5-year mortality rate of 39-68 percent<sup>2,3</sup>. During a 2-year period, the medical costs for a single diabetic patient with a foot ulcer are estimated at approximately \$28,000 and this figure would rise considerably in the case of continued care or amputation<sup>4,5</sup>.

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*Correspondence to: Daniela Anna Cutrignelli, Department of Plastic and Reconstructive Surgery, National Cancer Institute "Giovanni Paolo II", Viale Orazio Flacco 65, 70124 Bari, Italy (e-mail: [daniela.cutrignelli@gmail.com](mailto:daniela.cutrignelli@gmail.com))*

Treatment of progressive necrotizing infection in the diabetic foot is extremely challenging. Therefore, prompt and effective treatment would prevent major complications and amputation. The current standard treatment for foot ulcers consists of accurate debridement, treatment of the infection, pressure off-load and arterial revascularization, if required <sup>6</sup>. However, despite an optimum standard care, the wound healing rate in large ulcers is poor.

In the last decade, several different treatments have been developed and evaluated to improve healing. Among these, living skin equivalents have been shown to be reliable, effective and safe <sup>7</sup>.

Hyalograft 3D (Fidia Advanced Biopolymers, Abano Terme, Italy) is a living dermis equivalent, onto which autologous human fibroblasts are isolated and propagated for subsequent passaging for a 14 day period. The cells are then seeded on a three-dimensional non woven biodegradable scaffold composed entirely of a benzylic ester of hyaluronic acid <sup>8</sup>.

The bio-engineered dermis provides immediate coverage, integrates with the ulcer and differently from an autologous skin graft, promotes healing by providing a much higher concentration of new healthy fibroblasts which release growth factors and cytokines<sup>9</sup>.

A retrospective matched Case-Control study was performed to assess the effectiveness of this new treatment based on the association between autologous fibroblasts and vacuum-assisted closure (V.A.C.) therapy in comparison with the standard care.

## Material and Methods

Between January 2000 and December 2004 we had 1050 referrals to our diabetic foot clinic, seventy of whom had a large infected foot ulcer. A retrospective matched Case-Control analysis was performed to compare the standard care with a new treatment based on bio-engineered dermis (Hyalograft 3D) and vacuum-assisted closure therapy (V.A.C.; KCI, Inc., San Antonio, Texas), to treat extensive necrosis of the foot. Inclusion criteria were diabetes type I or II; age >18; acute infected necrosis of the foot (present for < a week); tendon and/or bone exposure (Wagner III and IV) after the first debridement; ulcer size  $\geq 30$  and  $80 \leq$  cm<sup>2</sup> on the plantar and/or dorsal surface of the foot and ability to deambulate before ulcer onset. Exclusion criteria was ABI index  $\leq 0.8$  (with or without previous arterial revascularization); TcPO<sub>2</sub>  $\leq 30$  mmHg; undergoing treatment of oral or parenteral corticosteroids, immunosuppressive or cytotoxic agents, coumadin or heparin; history of bleeding disorders; evidence of osteomyelitis on X-Ray, which incidentally, represented the main factor for exclusion. Only 20 patients (9 ♂ and 11 ♀, aged 42-80, mean age 65.5) met the criteria and entered the study (Table I). Due to the site and entity of the ulcer, none of these patients was able to deambulate without aid.

As shown in Table II, patients were divided into two homogeneous groups of ten patients each, depending on the treatment they had undergone, the choice of which was totally random and influenced solely by the availability of Hyalograft 3D in our clinic.

All the patients were stabilized from a metabolic point of view. This involved restoration of the fluid and electrolyte balance, correction of hyperglycemia, hyperosmolarity, acidosis and azotemia and treatment of exacerbating disorders. Surgical debridement was performed within the first 24 hours following the first visit to the clinic and the majority of the patients received the first treatment on the same day of admission (within 12 hours of the first referral to the clinic).

The control group was treated with the standard method based on surgical debridement, moist dressings, off-load and autologous skin grafts as described in Table III.

TABLE I - Baseline characteristics of the population

	Population (20 patients)
Age	42-80 (mean 65.5 y.o)
Sex	9 ♂ 11 ♀
Wound Size (cm <sup>2</sup> ; mean $\pm$ SE)	56.45 $\pm$ 2.7
ABI Index (mean $\pm$ SE)	0.874 $\pm$ 1
TcPO <sub>2</sub> (mmHg; mean $\pm$ SE)	46.9 $\pm$ 1.4
Revascularization	6

TABLE II - Characteristics of the two groups before the treatment

	Control Group (10 patients)	Study Group (10 patients)	P
Age	42-72 (mean 67 y.o.)	46-80 (mean 65 y.o)	
Sex	6 ♂ 4 ♀	3 ♂ 7 ♀	NS
Wound Size (cm <sup>2</sup> ; mean $\pm$ SE)	57.6 $\pm$ 4.5 cm <sup>2</sup>	55.3 $\pm$ 3.2 cm <sup>2</sup>	NS
ABI Index (mean $\pm$ SE)	0.873 $\pm$ 1.5	0.876 $\pm$ 1.5	NS
TcPO <sub>2</sub> (mmHg; mean $\pm$ SE)	48.1 $\pm$ 1.9	45.7 $\pm$ 2	NS
Revascularization	3	3	NS

TABLE III - Therapy protocols

DIABETIC FOOT PATIENTS		Control Group
Study Group		
Day 1-21	- skin biopsy for autologous fibroblasts culture (Hyalograft® 3D). - multiple surgical debridements (three to seven). - advanced silver coated dressings - culture swabs once a week	- multiple surgical debridements (three to seven). - advanced silver coated dressings or Alginates. - culture swabs once a week
Day 22	1 <sup>st</sup> Autologous tissue graft	- Split skin graft as soon as the wound bed is prepared.
Day 29-35	VAC therapy (changed every 48-72 hours).	
Day 36	2 <sup>nd</sup> Autologous tissue graft.	
Day 43-49	VAC therapy (changed every 48-72 hours).	
Day 50-56	Split skin graft.	

The Study group was treated with a new method based on an association of bio-engineered dermis (Hyalograft 3D Autograft in thin sheets of 8x8 cm) followed by V.A.C. therapy and autologous split skin graft.

*The procedure was as follows:*

Day 1: skin biopsy (1-2 cm<sup>2</sup>, thickness 0.8 cm) for autologous fibroblast culture was taken from the arm or forearm and sent to the Tissue Tech® Laboratory for fibroblast cell culturing (FIDIA Advanced Biopolymers, Abano Terme, Italy) which took approximately 21 days to complete for each patient.

Day 1-21: multiple surgical debridements (3 to 7, depending on the extent of the necrosis and the response to the medical therapy). Advanced dressings with nanocrystalline silver (Acticoat; Smith & Nephew plc, London, England) after each debridement. A culture swab was taken weekly to evaluate any change in the

colonization of the wound and the antibiotic therapy was modified accordingly.

Day 22: 1<sup>st</sup> Autologous tissue-engineered graft (Hyalograft® 3D Autograft 8x8 cm). The number of sheets of Hyalograft-3D used depended on the size of the wound. The application of the engineered tissue was performed with sterile forceps to create a single layer of tissue, avoiding the overlapping of the sheets (Fig. 1). A non-adherent dressing was subsequently applied in contact with the graft followed by the application of an outer dressing (sterile cotton gauze and semicompressive elastic bandage) and left intact for 7 days. In cases of excessive oozing, the outer layer of the dressing was changed. Day 29-35: V.A.C. therapy 7 days after the tissue-engineered graft. The outer dressing was removed carefully to avoid damage to the graft. The wound was then gently washed with saline solution. V.A.C. foam was placed on the top and set at a negative pressure of



Fig. 1: Application of the autologous tissue-engineered graft over the wound after surgical debridement.



Fig. 2: V.A.C. Therapy 7 day after the tissue-engineered graft. V.A.C. foam was placed on the top of the wound and set at a negative pressure of -125 mmHg.



Fig. 3: Wound bed ready to be grafted after the removal of the V.A.C. Foam.



Fig. 4: Application of the split skin graft over the wound bed.

-125 mmHg (Fig. 2). The V.A.C. foam was changed every 48-72 hours for a maximum of 7 days.

Day 36: Both the V.A.C. dressing and the paraffin sterile dressing were removed. A careful debridement was carried out and the Hyalograft® 3D graft, as previously described, was performed.

Day 43-49: Vac therapy 7 days after the 2<sup>nd</sup> Hyalograft® 3D graft. The V.A.C. foam was again kept for a maximum of 7 days as previously described.

Day 50-56: The Split Skin Graft (meshed 1:3 or unmeshed) was applied after minimal surgical debridement (Figs. 3, 4).

Both the study and control protocols were rigorously carried out by the same team of four plastic surgeons. The parameters measured to assess the differences

between the two protocols were as follows: the healing time and rate, the percentage of graft take for each group, the recurrence rate after treatment, the percentage of patients able to deambulate at the 6<sup>th</sup> month follow-up, the incidence of major amputations and the death rate.

#### STATISTICAL ANALYSIS

Data were reported as mean ± standard error (SEM) and percentages. Comparisons of clinical and biochemical parameters between groups (“study” vs. “control”) were performed using the Mann Whitney U and the Fisher’s Exact tests. Statistical tests were conducted as a two tailed hypothesis with an alpha value of 0.05 and were seen to have a reasonable significance. All the statistical analyses were performed with NCSS software (NCSS, Kaysville, UT, USA, www.ncss.com).

#### Results

No differences were found between the two groups in terms of graft take, major amputations and deaths, while significant differences were observed concerning the percentage and time of healing, recurrence rate and deambulation at the 6<sup>th</sup> month follow-up (Table IV). The mean follow-up was 20 months (range 12-24).

**Control group:** In this group we were able to perform split skin grafts in 7 patients but were unable to perform any skin grafts in three patients, as the wound bed was not ready. Out of the 7 skin grafted patients, only 2 patients (28.6%) healed completely within the 20<sup>th</sup> week. Of the remaining 5 patients of the subgroup the skin graft healed between the 24<sup>th</sup> and 36<sup>th</sup> week. Minimal signs of infection were not taken into account as they were considered part of the healing process of a large wound. Two patients, one skin grafted within the 20<sup>th</sup> week and one not skin grafted within the study period, developed severe infections that required multi-

TABLE IV - Differences shown in the two groups

	RESULTS		
	Control Group	Study Group	P
Healing(%) at the 20 <sup>th</sup> week	28.6%*	90%	< 0.001**
Healing time (weeks)	27.6 ± 3.1	15 ± 1.5	<0.01**
Graft take (%) at the 20 <sup>th</sup> week	58.3 ± 12.1	80.5 ± 3.5	NS
Recurrence rate at 24 <sup>th</sup> month	100%	20%	< 0.001**
Deambulation at 6 <sup>th</sup> month	20%	80%	< 0.05**
Major Amputations	0	0	NS
Death	0	0	NS

\*The percentage refers to the 7 skin-grafted patients

\*\*Significant results



Fig. 5: Patient of the study group. a) large diabetic ulcer involving dorsum and plantar areas of the left foot with tendon and bone exposure; b) 6 month follow-up.

ple debridements, aggressive multi-antibiotic therapy, change of dressings twice a day for two weeks and minor amputations. Although none of the patients required major amputations, 8 patients were still not able to walk without aid during the study period and at the 6 month follow-up because of unhealed wounds. The two healed patients both developed a recurrence during the two year follow-up. All patients survived during the two year follow-up.

**Study group:** In this group we had a 90% rate (nine patients out of ten) of complete healing at the 20<sup>th</sup> week (Fig. 5). We had only one delayed healing due to infection (at the 24<sup>th</sup> week). However, none of the patients required major amputations. The remaining 9 patients healed within the 20<sup>th</sup> week and were able to walk without a walking aid at the 6 month follow-up.

Six patients developed new ulcers in the same or contralateral foot, but only 2 patients (20% recurrence) developed a recurrence in the treated areas and required further treatment. No side effects due to Hyalograft 3D Autograft were observed.

## Discussion and Comments

Clinical and experimental evidence suggests that diabetic ulcers and other types of chronic wounds experience wound healing impairment. Parts of the chronic wound lose the ideal synchrony of the phases that lead to rapid healing<sup>10</sup>. In the case of diabetic ulcers, this is caused by several intrinsic factors (neuropathy, vascular problems, associated diseases) and extrinsic factors (wound infection, callus formation, and excessive pressure to the site).

The standard treatment, according to the American Diabetes Association<sup>11</sup>, consists of debridement of necrotic and infected tissue, use of moist dressings, topical antiseptic, antibiotic therapy (oral or i.v.) and the use of a pressure off-loading device.

However, in recent years, grafts made from autologous fibroblasts grown on scaffolds made of the benzyl ester of hyaluronic acid have shown excellent results<sup>12-14</sup>.

Laboratory data suggest that bio-engineered autologous dermal substitute provides healthy living human dermal fibroblasts that deposit matrix proteins, facilitates neoangiogenesis, secretes a mixture of growth factors in physiological concentrations essential for wound healing and epithelialization<sup>15-16</sup>. An added advantage of autologous fibroblasts is that they do not initiate any immune response as can happen with allogenic human dermal substitutes<sup>17</sup>.

Falanga et al<sup>18</sup> also sustained that fibroblasts recruit stem and progenitor cells to the wound site. Uccioli et al<sup>8</sup> reported, in a large observational retrospective study on 401 diabetic foot ulcers using Hyalograft 3D as an autologous dermal substitute, a 70% healing rate in a year, 63% of which within 4 months, and a 11% recurrence rate at the one year follow-up.

Veves et al<sup>19</sup>, in a randomized 12 week trial of 208 patients with neuropathic ulcers, demonstrated that the use of a bilayered scaffold comprising living fibroblasts and keratinocytes from neonatal foreskin led to complete wound closure in 56% of the patients, compared with 38% in controls.

However, Caravaggi et al<sup>6</sup> in evaluating the effectiveness of Hyalograft 3D Autograft in non infected diabetic plantar and dorsal foot ulcers, found no difference between the engineered tissue and standard care in plantar ulcers when associated with a correct off-load. Although, a significant difference was found in the healing of dorsal ulcers compared to the standard treatment. As far as negative pressure wound therapy is concerned, Armstrong et al<sup>20</sup>, in a randomized controlled trial using the V.A.C showed that a more rapid healing occurs in complex postoperative wounds in the diabetic foot when compared with standard treatment alone.

Braakenburg et al<sup>21</sup> also reported that patients treated with vacuum-assisted closure healed 33% faster than patients in the group treated with advanced dressings. The difference between the standard care and the V.A.C. therapy became more evident for diabetic and cardiovascular patients whose wound-healing time was significantly lower in the V.A.C. group compared with the control group (14 days *vs* 23 days, respectively).

However, Argenta and colleagues<sup>22</sup>, in a group of diabetic patients treated with V.A.C. therapy, showed an 83% reduction in complications, but no significant effect on wound healing time at one year, compared to the standard care.

Interestingly enough, few studies have assessed the role of bio-engineered tissue associated with V.A.C. therapy<sup>23</sup>

which we believe, improves the growth rate of the fibroblasts. In fact, the V.A.C. seals the wound following the fibroblast graft and provides a moist environment for the tissue to survive as perfusion is increased and extra fluids are removed. Granulation tissue rapidly forms filling up the wound.

Oyibo et al<sup>24</sup> studied the healing rate of 194 patients with diabetic ulcers treated using standard procedures. A healing rate of 0.2 mm<sup>2</sup>/day was calculated. The size of the ulcer area was correlated with healing time and predicted healing, while patient's age, sex, duration/type of diabetes, and ulcer site had no effect on the outcome. In the same way, Zimny et al<sup>25</sup> assessed the wound size reduction and healing time in neuropathic, neuroischemic and ischemic diabetic foot ulcers by calculating the daily wound radius reduction. The wound healing rate was 0,045mm/day in neuropathic foot ulcers, 0.019 mm/day in the neuroischemic group and 0.0065mm/day in the peripheral occlusive vascular disease group.

On the contrary, given an average ulcer size of 55.27 cm<sup>2</sup> and a cut-off time of 20 weeks, our study showed an improved healing rate of 5.4 mm<sup>2</sup>/day using autologous fibroblasts associated with vacuum-assisted closure therapy.

Although we did not compare the healing rate of small ulcers<sup>25-26</sup> with larger ulcers, we feel our study gives a clear idea of the reduced healing time which can be achieved with Hyalograft 3D in association with V.A.C. therapy, in comparison with the standard care.

Although some authors<sup>2</sup> suggest changing from standard care to an appropriate advanced treatment should only be considered when an ulcer shows a less than 10-15% decrease in wound volume per week over a 3 week period, we strongly disagree with this idea as delayed treatment could result in the degeneration of the wound into a senescent state which could become irreversible.

In the study group, theoretically, the 21 day period needed to grow the autologous fibroblasts on the scaffold could be considered a limitation to our procedure as it increases the healing time. However, this is not the case, because the control group required about 21 days to complete the standard debridements before the grafting anyway.

Controversies still exist regarding the recurrence rate. In our experience, we found a reduced recurrence rate in the study group which could be related to the improved neoangiogenesis of the neo-dermis formed by the autologous fibroblasts and to the repopulation of the wound with much healthier cells, which are consequently more resistant to stressors.

The most difficult factor to assess is the device which is responsible for the higher healing rate. In diabetic patients with large infected ulcers, we did not obtain encouraging results in the use of V.A.C. therapy only (unreported data based on clinical observation). Therefore, we strongly believe that it is the association of the bio-engineered tissue and V.A.C. therapy which

is more effective than the devices used separately. Argenta et al.<sup>22</sup> reported a very rapid tissue growth when applying the vacuum-assisted closure device onto the Integra (INTEGRA® Dermal Regeneration Template Single Layer, SIAD Healthcare S.p.A.). We hypothesise that it is the negative pressure which boosts the growth of the fibroblasts, encouraging the production of growth factors and, subsequently, stimulating the neoangiogenesis which allows a faster growth of the neo-tissue and a higher graft take.

To confirm these hypotheses, the levels of growth factors and the characteristics of the fibroblasts under negative pressure should be studied further, as hypertrophy and hyperplasia of the fibroblast could be the answer.

Hyalograft 3D is an expensive product. If, however, it can reduce the need for surgical intervention, particularly amputation, it may be worth considering. On the whole, we believe that the reduction of nursing time, surgical procedure, change of dressings and healing time could make the use of Hyalograft 3D cost-effective.

Due to the retrospective nature of the study and the low number of patients involved, this can be considered as a preliminary report of a larger prospective study that we will publish in the near future.

## Conclusions

In conclusion, our analysis has highlighted a reliable, safe and cost-effective method of treating extensive necrotising infections of the diabetic foot.

Although further evaluations are needed, we strongly believe that bio-engineered dermis associated with vacuum-assisted closure device is a key factor for the treatment of large soft tissue defects in diabetic patients.

## Riassunto

La prevalenza mondiale del diabete nel 2000 è stata stimata essere del 2.8% con una previsione del 4.4% nel 2030. A causa della neuropatia e dei processi ischemici che ne derivano, i pazienti diabetici sono purtroppo predisposti all'insorgenza di infezioni a carico del piede con un rischio di amputazione molto elevato. A tal proposito, un trattamento rapido ed efficace del processo necrotizzante impedirebbe complicanze maggiori e la stessa amputazione.

Il nostro studio consiste in un'analisi retrospettiva condotta su 20 pazienti atti a valutare l'efficacia del sostituto dermico autologo bioingegnerizzato (Hyalograft 3D Autograft) in associazione alla V.A.C (vacuum assisted closure) Therapy per il trattamento delle ulcere diabetiche, rispetto ai metodi sinora impiegati.

I pazienti selezionati, a seconda del trattamento eseguito, sono stati suddivisi in due gruppi omogenei di dieci pazienti ciascuno. Il gruppo controllo è stato trattato

con il metodo standard, ovvero con debridement chirurgico e innesti cutanei autologhi, mentre il gruppo studio è stato trattato con il metodo in analisi.

La raccolta dei dati ha suggerito come l'impiego combinato del sostituto dermico bioingegnerizzato e della pressione negativa determini, rispetto al gruppo controllo, un aumento della percentuale di guarigione, una riduzione del tasso di recidiva di lesioni anche estese e una maggiore ripresa della deambulazione autonoma.

In conclusione, la nostra analisi ha evidenziato un metodo sicuro, affidabile e competitivo per il trattamento di ampie lesioni necrotizzanti del piede diabetico. Anche se ulteriori valutazioni sono necessarie, crediamo fortemente che l'associazione del derma bioingegnerizzato e della pressione negativa possa condurre ad una rapida ed efficace guarigione delle ulcere, migliorando la qualità della vita del paziente diabetico.

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