Factors Influencing Efficacy of Bilayered Cell Therapy

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Objective: Diabetic foot ulcers (DFUs) that fail to heal with standard care should be treated with advanced wound care products. Efficacy of advanced therapies is dependent on many factors. A secondary analysis of pivotal trial data for a bilayered cellular construct used in the treatment of DFU was undertaken to determine if glycemic control and other factors had an effect on time to healing.

Approach: We analyzed the effect of age, gender, diabetes type, insulin usage, body mass index, smoking, initial and ending glycohemoglobin (HgbA1c), Charcot deformity, and wound area, duration, and location on likelihood of healing for wounds treated with bilayered cellular construct (BLCC).

Results: In those treated with BLCC, initial wound area (cm²), age, and history of Charcot deformity were found to significantly affect healing. Neither initial HgbA1c nor change in HgbA1c was associated with healing. The bilayered product was found to be equally effective regardless of initial or change in HgbA1c levels (*p*-values 0.94 and 0.44, respectively). In the control group, initial HgbA1c, insulin usage, female gender, and wound location at the toes significantly influenced healing.

Innovation: BLCC subgroup analysis to elucidate selection criteria allowing for targeted use of advanced products on those more likely to respond as well as direct further research into prognostic indicators for BLCC-treated patients.

Conclusion: The bilayered cellular construct product remains equally effective regardless of initial or change in HgbA1c levels. Further specific research into the effect of glucose control and other factors on the effectiveness of different advanced DFU treatment products is recommended.

INTRODUCTION

DESPITE INCREASING AWARENESS of treatment modalities for diabetic foot ulcers (DFUs), healing rates remain unacceptably low and resultant amputation rates remain high.¹ Initial treatment consists of assessment of vascular sufficiency and wound, soft tissue and bone infection, and subsequently offloading and debridement.² Should treatment fail, adjuvant treatment is recommended. Two cell-based products are currently FDA-approved for the treatment of recalcitrant DFU:

a human dermal fibroblast (HDF) substitute (Dermagraft; Organogenesis, Canton, MA) and a bilayered cellular construct (BLCC, Apligraf; Organogenesis). Although studies evaluating the factors associated with success with the use of these products are limited, interest in the importance of glycemic control as a modifiable potential factor and its association with healing in the chronic DFU has been investigated.^{3,4} The focus has been on glycohemoglobin (HgbA1c), the most commonly used indicator of

3-month average glycemic levels in patients with diabetes mellitus (DM). In a retrospective cohort study, Christman *et al.*³ found that for each 1.0% (11 mmol/mol) increase in HgbA1c, the daily wound area healing rate decreased by 0.028 cm² per day ($p=0.027$). However, the consideration of the effect of HgbA1c on healing rates with adjuvant treatment was not assessed. Marston *et al.*⁴ analyzed the influence of glycemic control on wound closure by the treatment group. They conducted a secondary analysis of a randomized controlled study ($n=245$) to examine risk factors for wound healing in patients enrolled in the HDF-substitute group and control group. They found that increasing HgbA1c levels (poor glycemic control) during the time course of the study affected patients' results: HDF-treated subjects with increasing HgbA1C did no better than control treatment (19% wound closure, p -value not reported), whereas HDF-treated subjects with decreasing HgbA1c had improved wound closure compared with control (46.7% wound closure, $p=0.009$).

CLINICAL PROBLEM ADDRESSED

Factors (including glucose control) that affect BLCC efficacy are largely unknown, as are the factors that affect standard of care treatment efficacy for refractory DFU.⁴ To elucidate possible prognostic factors for success with BLCC treatment, statistical subgroup analysis of previously published pivotal trial data for BLCC was utilized.

MATERIALS AND METHODS

Device description

BLCC is a bilayered living cell therapy consisting of a bovine collagen lattice with embedded human, neonatal, foreskin-derived cultured fibroblasts. This collagen/fibroblast lattice is covered with a layer of human cultured keratinocytes to produce the final bilayer product. Fibroblasts and keratinocytes secrete growth factors and cytokines necessary for healing resulting in neoangiogenesis, native matrix deposition, native fibroblast and keratinocyte proliferation and migration, and ultimately healing.⁵

BLCC pivotal trial

Details have previously been published, including inclusion/exclusion criteria and patient characteristics.⁶ Briefly, it was a multicenter, randomized, single-blinded, controlled trial comparing the BLCC product with a control regimen of saline-moistened gauze and petrolatum gauze. All ulcers were clinically free of infection and under-

went sharp debridement. All patients received standardized offloading devices and were non-weight bearing for the first 6 weeks. BLCC was placed at day 0, and weeks 1–4, with a maximum of five applications during the 12-week trial. Tracings and photographs were obtained weekly on all patients. Characteristics of the control and study groups are shown in Table 1, and no significant differences were found at baseline. Primary endpoint was percent of wounds 100% healed (wound closure) at week 12. Fifty-six percent of the bilayered-treated group and 38% of the control-treated group healed ($p=0.0042$). Additionally, median time to closure was analyzed via Kaplan–Meier analysis (65 days for BLCC vs. 90 days for control, $p=0.0026$), and multiple regression for factors affecting time to closure was analyzed (adjusted hazard ratio [HR] for healing 1.59 for BLCC over control, p not reported). Subgroup analysis was not reported.

Current study subgroup analysis

Individual level data for the BLCC-treated patients were obtained from the initial trial database (BLCC $n=112$ of 208 enrolled). Since the possible predictor variables were numerous and mixed in nature (categorical and continuous), as well as to control for possible confounding effects, a regression model was needed. Additionally, these data were essentially a time-to event (healing) study. Thus, survival analysis techniques rather than logistic multivariable regression were elected. Data were analyzed using the Cox Proportional Hazards Model, with an endpoint of wound closure (100% epithelialization), similar to the original pivotal trial. Cox model results are reported as HR for each variable analyzed with corresponding p -values. The HR can be roughly interpreted as the relative likelihood (approximate% increase or decrease) of

Table 1. Characteristics of the control and treatment groups

Characteristic	BLCC, $n=112$	Control, $n=96$
Age at treatment	58 ± 10	56 ± 10
Diabetes duration (years) ^a	15 ± 10	13 ± 10
Wound duration (months)	6 ± 13	6 ± 12
BMI (kg/m ²)	31 ± 6.5	33 ± 7.7
Wound area (cm ²)	2.97 ± 3.1	2.83 ± 2.45
Baseline HgbA1c (%) (mmol/mol)	8.6 (70) ± 1.5 (16.4)	8.6 (70) ± 1.4 (15.3)
Change in A1c ^b	−0.17 ± 1.3	−0.03 ± 1.2
Charcot	17 (15)	22 (23)
Gender (M/F) (%)	88/24 (79/21)	74/22 (77/23)

No significant differences were found by t -test.

Data are mean ± SD or n (%).

^a $n=111$.

^b $n=93$ BLCC and 75 control.

BLCC, bilayered cellular construct; BMI, body mass index; HgbA1c, glycohemoglobin.

the chance of the event, in this study, the event is healing (e.g., a HR of 1.21 for variable X implies a 21% greater chance of healing for subjects with trait X). The statistical significance of a reported HR is determined by the corresponding p -value. In this study, we considered a p -value of <0.05 to be significant and a value of 0.05–0.2 to be trending. Each variable was addressed separately in single variable (univariate) analysis as well as multivariable-adjusted models for final results. The statistical assumptions of the Cox model were tested, and no gross violations of the proportional hazards model assumptions were observed for any of the variables.

Statistical software

Regression and statistical analyses were performed using R v 2.14.0, a statistical software program developed and distributed by the R Project for Statistical Computing, freely available on the Internet.

Predictor selection and transformation

We evaluated the following predictors: age, gender, investigational site, body mass index, smoking history, current smoking status, diabetes type, diabetes duration, wound duration, wound area, presence or absence of Charcot deformity, baseline HgbA1c, week-12 HgbA1c, change in HgbA1c levels during the trial, and location of the wound (toes, forefoot, and midfoot)^{3,7–9} Margolis *et al.* have shown that a log transformation may be appropriate for certain predictors, particularly wound area and wound duration, given their usually significantly skewed distributions.^{8,9} Because skewed distributions were found for those predictors in this trial data, models using both the log transformed and raw values for wound area and duration were studied. Despite increasing the significance of wound area p -values (lower values), log-transformation did not affect the results in terms of overall statistically significant factors in final models. Because interpretation of the analysis results is simpler and clinically more relevant for untransformed predictors, p -values and HR for untransformed wound area and wound duration are reported.

Change in HgbA1c values was modeled as a time-dependent variable for the Cox model, since in those patients in whom the wound healed, the measurement of 12-week HgbA1c occurred after the event of healing. In addition, including the raw change in HgbA1c (12 week – initial HgbA1c) in the analysis is redundant, given the presence of initial and 12-week numbers as variables. Initial HgbA1c and change in HgbA1c were also analyzed as categorical variables.

Inclusion of a factor in final adjusted multivariable model was determined using several criteria, including clinical significance/sense, statistical significance (likelihood ratio p -value <0.05), coherence with the proportional hazard model assumption, minimizing the corrected Akaike information criterion, as well as maximizing the R^2 of final models.

RESULTS

We evaluated 91 of 112 BLCC-treated patients as a complete data set (week 12 HgbA1c diabetes duration, wound location was missing for some). The analyzable subgroup of patients did not show significant deviations from the overall study group characteristics in Table 1.

Neither baseline, 12-week HgbA1c, nor change in HgbA1c had a significant effect on likelihood of healing in the BLCC-treated group in either single variable or multivariable models (Cox model results in Table 2). In adjusted multivariable time-to-healing analysis, baseline wound area (HR=0.77, $p=0.003$), patient's age (HR=0.01, $p=0.01$), and Charcot status (HR=0.03, $p=0.003$) were found to be important predictors of response to the BLCC. A strongly significant interaction term was noted between the presence of Charcot and wound area (HR=1.7, $p=0.002$). All other variables were not significant except for a trend toward nonhealing in smokers (HR=0.53, $p=0.12$) and longer wound duration (HR=0.98, $p=0.08$) (Cox model results in Table 2).

For the control group, we found initial HgbA1c (HR=1.36), insulin usage (HR=0.35), gender (fe-

Table 2. BLCC group single variable and multivariable model results

Variable	BLCC Group Analysis			
	Univariate		Multivariable	
	Hazard Ratio (95% Limits)	p-Value	Hazard Ratio (95% Limits)	p-Value
Gender (female)	1.67 (0.95–2.94)	0.08	—	—
BMI	0.98 (0.94–1.02)	0.40	—	—
Smoking	0.81 (0.37–1.77)	0.60	0.53 (0.23–1.19)	0.120
Age	0.98 (0.96–1.01)	0.16	0.96 (0.94–0.99)	0.010
Diabetes type (type I)	0.94 (0.38–2.35)	0.89	—	—
Insulin usage	0.66 (0.4–1.11)	0.12	—	—
Baseline A1c	1.01 (0.86–1.18)	0.94	—	—
Change in A1c ^a	1.12 (0.84–1.50)	0.44	—	—
Wound duration (years)	0.97 (0.94–0.99)	0.01	0.98 (0.95–1)	0.077
Wound area (cm ²)	0.86 (0.76–0.97)	0.01	0.77 (0.65–0.92)	0.003
Location midfoot ^b	0.49 (0.26–0.91)	0.02	—	—
Location toes ^b	1.31 (0.72–2.38)	0.37	—	—
Charcot	0.18 (0.06–0.57)	0.004	0.03 (0–0.31)	0.003
Charcot: area interaction	—	—	1.7 (1.22–2.38)	0.002

^aModeled as time-dependent variable.

^bReference location metatarsal heads.

male, $HR=0.34$), and wound location at the toes ($HR=2.49$) all to be statistically significant ($p<0.05$).

Figures 1 and 2 present the effect of initial A1c levels and change in A1c levels (as categorical variables) respectively on wound closure by group.

DISCUSSION

The initial impetus for this study was to evaluate whether glycemic control had an effect on the ability of BLCC to heal refractory DFU. Neither baseline nor change in HgbA1c levels had a significant effect on outcomes ($HR\ 1.01$ and 1.12 , respectively). This is important because in patients requiring a cell-based therapy for their ulcers, the BLCC can be used without necessarily waiting for optimal glycemic control. Time to instituting care may be crucial factor for amputation avoidance. While benefits of glycemic control exist,¹⁰ delay of BLCC pending an improving trend in HgbA1c levels is not necessary.

Why BLCC is not affected by glycemic control remains unclear. Cellular dysfunction of the diabetic fibroblast in a hyperglycemic environment has been documented by Lerman *et al.*¹¹ However, in a study on the effects of glucose on keratinocyte function, both keratinocyte differentiation and response to keratinocyte growth factor were enhanced in a high glucose environment.¹² Thus, this may explain why the BLCC is unaffected by glucose levels—as enhancement of the keratinocyte function may overcome fibroblast dysfunction in high glycemic environments. We did find that a larger wound area affected the time to healing in BLCC-treated patients ($HR=0.77$, $p=0.003$). This is not surprising, and a full discussion of the interplay between wound area, location, and wound duration ($HR=0.98$, $p=0.08$) as predictors of healing has been reported in detail.⁹

We also found that smoking was associated with a decreased likelihood of healing ($HR=0.53$, $p=0.12$). While this did not reach statistical significance, it is of importance as to date, no studies have shown a detrimental effect of smoking on wound healing in DFU.

We also found that history of Charcot deformity affected healing and is consistent with the finding that Charcot foot is a known risk factor for poor healing and amputation.¹³ Larger wound surface area and presence of Charcot deformity were associated with lower response to BLCC. However, a very strong interaction term was noted between Charcot and area ($HR=1.7$, $p=0.002$), indicating that Charcot wounds with larger area had a higher likelihood of healing than smaller wounds when treated with BLCC.

Figure 1. Healing percent by initial A1c and treatment group% (mmol/mol). Model analysis shows significant healing advantage of Apligraf treatment over control for all initial A1c levels ($p<0.0091$). Initial A1c was not a significant predictor of healing in the Apligraf group ($p=0.9385$). Higher initial A1c for the control group showed increased healing ($p=0.02$). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Important results are found in the control group. Significantly decreased likelihood of healing with insulin use and among women in the control group may indicate a need to be more aggressive in transitioning to advanced therapies when treating these patients. Further product-specific analyses may help in illuminating a rationale for choice of product in specific patient populations. Further evaluation of HgbA1c levels on specific product efficacy and evaluation of other modifiable factors not routinely assessed for in trial designs are warranted.

Study limitations: The main limitation of this study is the small sample size. Thus, the effect of some variables may have been masked or inflated due to too few subjects, specifically with respect to possible gender effects (only 24 females of 112 subjects, 21%), Charcot (only 17 of 112, 15%), and DM type (only 9 of 112 type I, 8%). Another limi-

Figure 2. Healing percent by change in A1c and treatment group. Change in A1c (increasing/decreasing) was not a significant moderator of Apligraf efficacy ($n=93$, $p=0.44$) or control ($n=75$, $p=0.29$). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

tation is the fact that the original study from which these data were extracted was not designed as a predictive trial. Thus, there may be as yet unknown factors that affect BLCC efficacy, which were not addressed in this analysis.

INNOVATIONS

BLCC subgroup analysis can elucidate selection criteria allowing for targeted use of advanced products on those more likely to respond as well as direct further research into prognostic indicators for BLCC-treated patients. This study shows that BLCC may be used effectively in diabetic patients regardless of glycemic control, a clinically relevant conclusion.

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KEY FINDINGS

- Factors affecting healing in chronic DFU remains only partially understood and may differ according to treatment product used.
- Treatment of refractory DFUs using BLCC is effective and independent of HgbA1c levels, and its application need not be delayed pending optimization of glycemic control.

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Abbreviations and Acronyms

BLCC = bilayered cellular construct
 BMI = body mass index
 DFU = diabetic foot ulcer
 DM = diabetes mellitus
 HgbA1c = glycohemoglobin
 HR = hazard ratio
 HDF = human dermal fibroblast