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HOLOGENIX

TCPO2 Testing
Overview and Procedures

1112 Montana Ave.●#13● Santa Monica, CA 90403

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Re: *Testing overview and procedures*

To Whom It May Concern:

At Hologenix, LLC we understand and appreciate the skepticism surrounding our technology and resulting claims. Accordingly, we have dedicated a significant amount of resources to develop, test and validate our science. Celliant's mineral matrix provides a wide range of benefits. To substantiate our claims and provide assurances to their validity, all Celliant products undergo extensive and methodical testing. This is performed in addition to independent clinical trials and studies. We have also established a Research and Development Laboratory, which has been approved to collect data for the purpose of generating original research for the peer-review process and publication. The Laboratory will work closely with the members of the Science Advisory Board, along with additional collaborators, to maximize its contributions.

In our lab, we utilize the same machine used in the clinical studies, a Perimed Periflux System 5000. This machine is widely recognized as the industry standard in the medical field when testing for $TCPO_2$. This system of measuring was initially developed to test for hypoxia, or low issue oxygen levels, in subjects suffering from diabetes and other circulatory disorders. It is also used to measure the effectiveness of diabetic medication.

The lead sensor is heated to 45°C to eliminate any increase in oxygen due to skin warming. The test lasts three hours and consists of a 90-minute period using a placebo garment followed by a 90-minute period with a Celliant garment. This allows each subject to serve as his/her own control. The subject is seated comfortably with vital signs such as blood pressure, heart rate, body temperature and grip strength taken at arrival, after 90-minutes, and then again at the conclusion of the test.

At Hologenix, we have the capacity to test 2-3 people per day, and to date we have tested over 200 subjects. These subjects are chosen at random using Craigslist.com. All healthy people age 18-70 are welcome. The only requirement is that they be a non-smoker and abstain from alcohol and caffeine the day of testing. We do not screen our subjects based on perceived health. We test every product containing Celliant from fabric to prototype including: shoes, socks, shirts, pants, jackets, mattress pads, and every other product before it is allowed to enter production. On average, there is an increase in $TCPO_2$ of approximately 7%. This significant increase is the catalyst for the benefits cited by Hologenix. To help generate further awareness and gain valuable feedback, we provide a pair of socks to each subject. Please visit facebook.com/Celliant for testimonials.

In conclusion, Hologenix believes in making sure each and every product meets our standards. We have also included a thermographic image of the placebo versus the Celliant to show that Celliant retains infrared energy.

Please direct any and all further questions regarding our testing procedure to our Testing Director, Trenton Horinek at trent.horinek@celliant.com, or by phone at +1 888-721-1545.

Thank you for your interest in our technology

Best regards,

Seth Casden

Seth Casden
CEO
Hologenix, LLC

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Re: Updated TCPO2 Findings

To Whom It May Concern::

My name is Michael Coyle and I am the Chief Science Officer for Hologenix, the maker of Celliant®.

As you may know, our technology is composed of finely ground, optically responsive minerals that are added to yarn to make garments. The minerals absorb infrared light emitted from the body and reflect it back into the skin and tissue, which results in the vasodilation of capillaries to improve circulation and tissue perfusion and oxygenation.

I would like to take this opportunity to share the findings of our latest $tcPO_2$ validation study in hopes that you will find the methods and statistical exactitude used to evaluate the data to be more than acceptable to substantiate any ambiguity with respect to Celliant efficacy.

The Principal Investigator of the clinical study was Ian Gordon, M.D., Ph.D., who is an Associate Clinical Professor of Surgery at the University of California, Irvine Medical School. Fifty-one healthy men and women were enrolled in the study that measured tissue oxygenation using TCPO2 as the primary endpoint.

The results of the study showed that, when compared to baseline, measures of tissue oxygenation were statistically greater when wearing a Celliant garment and, further, corresponded to a mean increase in tissue oxygenation of seven percent (7%).

Included is a detailed report of the study, methods and statistical analysis plan used to evaluate the data. We invite you to evaluate the study and look forward to answering any questions that you may have.

Kind regards,

Michael Coyle, Ph.D.

Michael Coyle, Ph.D.
Chief Science Officer
Hologenix, LLC

Transcutaneous Partial Pressure of Oxygen ($tcPO_2$) as a Primary Endpoint to Assess the Efficacy of Celliant® as a Vasoactive Material

INTRODUCTION

Celliant® technology is a patented process for adding micron sized optically active quartz, silicon oxide and titanium oxide particles to polymer fibers. The resulting Celliant® yarns have unique effects on the electromagnetic energy environment of the skin in the visible and near infrared portion of the spectrum leading to increased blood flow and oxygen levels in the tissue.

This report is a summary of data collected under the auspices of Ian Gordon, M.D., Ph.D., Associate Clinical Professor of Surgery at the University of California- Irvine Medical School. Fifty-one (51) healthy men and women were enrolled in the study. This study was a within subjects trial, which is noted for its ability to control for individual differences among subjects. Since each subject is assessed under each level of the independent variable or condition, the subjects serve as their own control, with the result that one of the largest sources of between treatment differences, inter-subject variation, is controlled (Keppel, 1991; Lindquist, 1953).

In the medical sciences, statistical significance levels are stated, *a. priori*, to know whether or not the treatment was efficacious relative to the control or baseline. A stringent statistical significance level (alpha = 0.05) is typically chosen so that if the study were to be reproduced, one would get the exact same results 95 times out of 100. Therefore, a p-value of *less* than 0.05 is perfunctory to meet this requirement. This represents a reasonable and realistic value for research in the medical and biological sciences (Cohen, 1965, 1977) and suggests that the likelihood is decent that a treatment effect will be detected, assuming a modest effect size (Chase & Tucker, 1976).

OBJECTIVES

The objective of this pilot study was to test the null hypothesis that a novel, optically active garment made with Celliant® (CL) material would not influence mean $tcPO_2$ over a 90-min period differently than when compared to a baseline (BL) period of the same duration (90-min).

PRIMARY ENDPOINT

The primary endpoint in this study was transcutaneous partial pressure of oxygen (tcPO2), measured in units of mmHg, which was used to assess treatment efficacy. This endpoint has been

used in numerous clinical trials and is a well-accepted clinical measure for tissue perfusion and oxygenation (Burgess, Matsen, Wyss, & Simmons, 1982; Dooley, Schirmer, Slade, & Folden, 1996; Franzeck, Talke, Bernstein, Golbranson, & Fronek, 1982; Hanna et al., 1997; Jaszczak, 1988; Le Devehat & Khodabandehlou, 1990; Le Devehat, Khodabandehlou, & Vimeux, 2001; Matsen, Bach, Wyss, & Simmons, 1980; Matsen et al., 1980; Matsi, Manninen, Suhonen, Pirinen, & Soimakallio, 1993; Shoemaker & Vidyasagar, 1981; White et al., 1982) with well established norms for intra-subject variability (Coleman, Dowd, & Bentley, 1986; Wagener & Hendricker, 1987)

MATERIALS AND METHODS

Subjects

Fifty-one (51) heathy men and women enrolled in the study (37 men; age 33.4 yrs (SD 9.3) and 14 women; age 37.2 yrs (SD 7.7). Subjects known to be active smokers (Fewings, Rand, Scroop, & Whelan, 1966; Mayhan & Patel, 1997) or engaged in recreational drug use for the six months prior to the start of the study were excluded. Patients were postprandial two (2) hours and refrained from alcohol ingestion (Altura & Altura, 1982; Fewings, Hanna, Walsh, & Whelan, 1966) within forty-eight (48) hours and caffeine ingestion (Umemura et al., 2006) within four (4) hours prior to testing.

Methods

Skin Preparation. Preparation of the subject was standardized to the following: the hair was shaved from the bicep of dominant arm; the dermis was then abraded with a fine abrasive material; the stratum corneum was then removed by the use of light weight adhesive tape; and finally, the probe site was wiped with an alcohol preparation swab.

Measurement of transcutaneous oxygen ($tcPO_2$). Subjects were seated in a comfortable chair. Room temperature was maintained at a constant temperature over the duration of the study. Baseline measurements (BL) of $tcPO_2$ were recorded for ninety (90) minutes at the bicep. During this time, the subject wore a standard shirt. After the baseline period, subjects donned a Celliant® shirt and subsequent measurements of $tcPO_2$ were recorded at the bicep for ninety (90) minutes. Transcutaneous partial pressure of oxygen ($tcPO_2$) data points were taken at t=10-min,

30-min and 90-min during BL and with CL. All measurements of transcutaneous oxygen tension were recorded using a PeriFlux System 5000 (Perimed, Inc., Kings Park, NY, USA) and modified Clarke Electrodes (Radiometer America, Inc., Ohio, USA). Data were sampled using Perisoft Version 2.10 (Perimed America, Inc., North Royalton, Ohio, USA).

All subjects received the same treatment in the same order: Baseline (BL) followed by Celliant® (CL). Transcutaneous oxygen tension ($tcPO_2$) does not vary significantly over time, therefore, establishing a baseline prior to measuring a treatment effect was warranted.

STATISTICAL ANALYSES

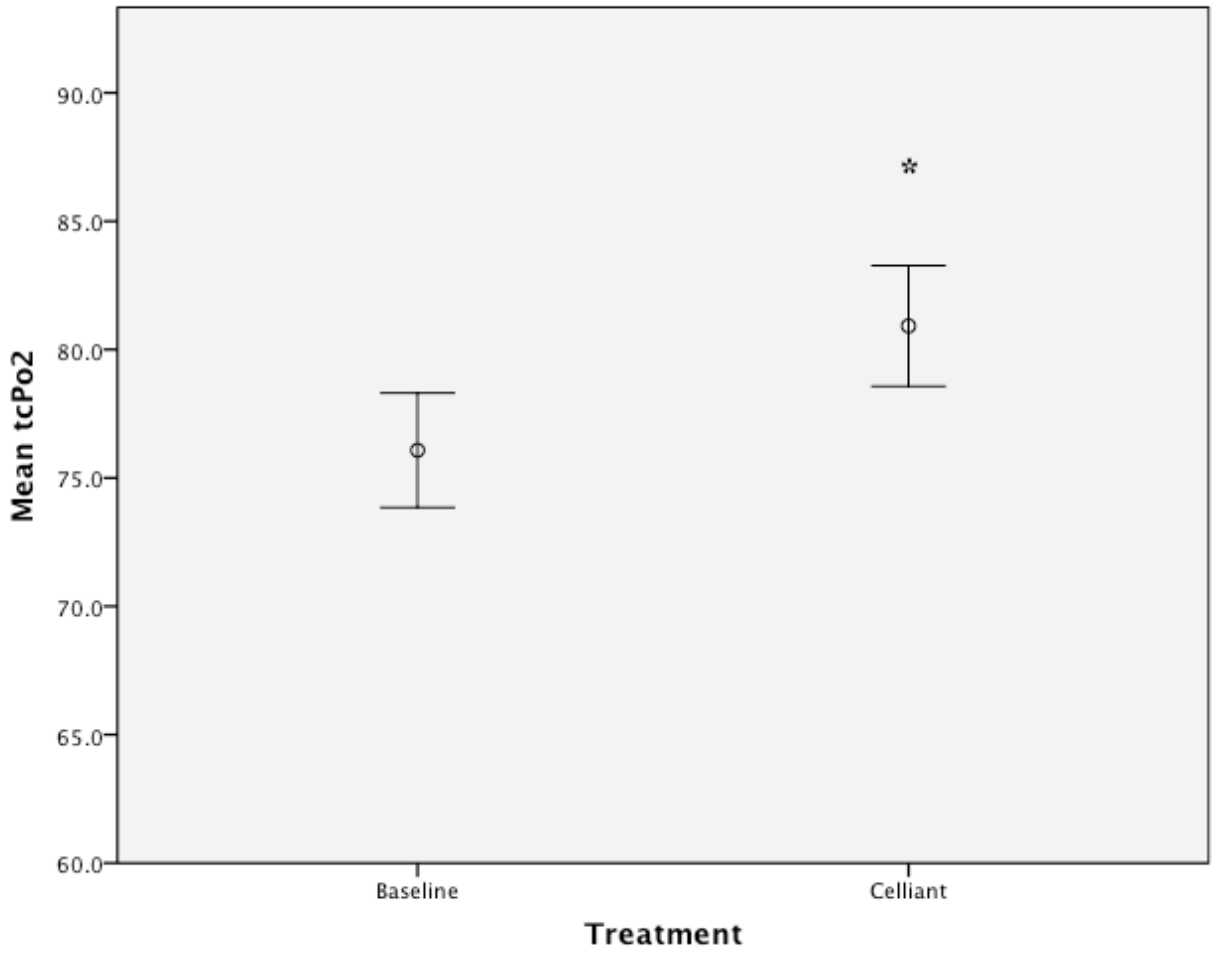
Continuous variables are summarized with standard descriptive statistics including means, standard deviations (SD) and 95% confidence intervals (95% CI). Inferential analyses were conducted using two-way repeated measures analyses of variance (ANOVA). All data were analyzed using SPSS (IBM, 2011). Statistical significance for this study was set at alpha = 0.05. Thus, a p-value < 0.05 was necessary to be considered statistically significant.

An analysis of the entire data set identified four (4) missing data points (three (3) data points from the Celliant® condition and one (1) data point from the Baseline condition). This resulted in a data set equal to 98.7% of the total expected data.

Multiple analyses were executed to evaluate the data. At the highest level, to evaluate whether or not the primary efficacy variable was sensitive enough to detect a difference between the Baseline and the Celliant® conditions, a one-way ANOVA was employed to test the means. The Celliant® treatment was statistically greater than the Baseline after ninety (90-min) (CL = 81.5 mmHg (SD 14.5), 95% CI [79.1, 83.7]; BL = 76.6 mmHg (SD 14.1), 95% CI [74.3, 78.9], $F(1, 294) = 8.602$, p. = 0.004. This represented a mean percent change from Baseline of seven percent (7%).

Figure 1 demonstrates the mean treatment difference between Celliant® and Baseline, as defined by the primary efficacy variable, $tcPO_2$, over the ninety (90) minute measurement period.

Figure 1. Difference in Treatments as Measured by tcPO2



Next, a two-way Repeated Measures ANOVA was employed to evaluate the influence of treatment at the different time points. The interaction between Condition (BL & CL) and Time was not significant ((F(1, 45) = 0.012, p. = 0.914). However, there was a significant within subject contrast for Time (F(1, 45) = 7.423), p. = 0.009). Pairwise comparisons were conducted using dependent sample *t*-tests to evaluate the differences between the means to identify statistical differences between specific time points. At all time points, the Celliant® condition was statistically greater than the Baseline as assessed by two-tailed, paired *t*-tests and

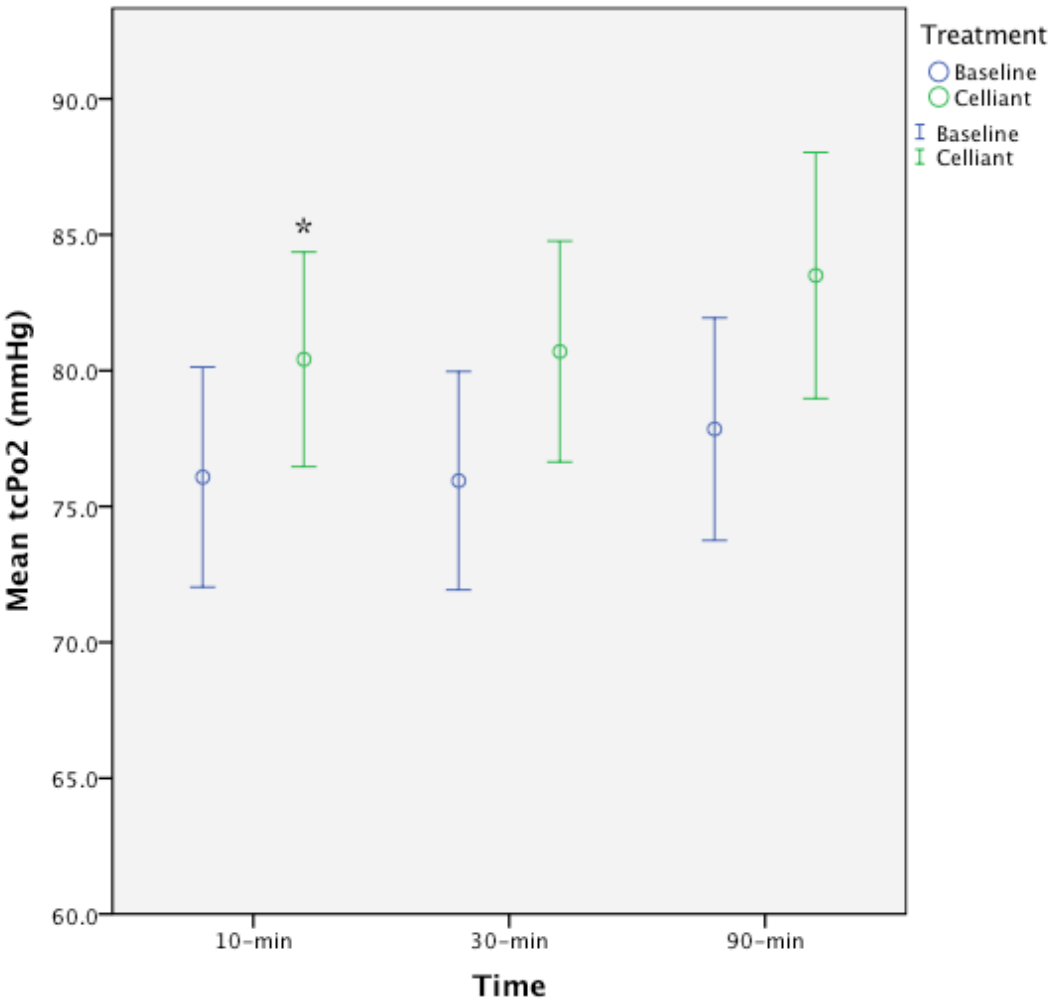
corresponded to a mean change from the Baseline condition of seven percent (7%). See Table 1 for a summary of these data.

Table 1. Summary of Two-tailed Paired *t*-tests vs. Time

Time	Mean CL tcPO2 mmHg	Mean BL tcPO2 mmHg	*t* statistic	d.f.	P-value
10-min	80.3	76.1	-2.60	50	0.012
30-min	80.7	75.9	-3.14	50	0.003
90-min	83.8	78.6	-3.22	46	0.002

To be certain that mean $tcPO_2$ did not significantly increase over time during the Baseline, as well as to show that the Baseline did not influence the Celliant® condition, a two-tailed paired *t*-test was executed in the Baseline condition between t=10-min (76.2 mmHg (SD 14.2)) and t=90-min (77.8 mmHg (SD 14.1), $t(49) = -1.18$, p. = 0.242. Figure 2 shows the difference in treatment means graphically.

Figure 2. Mean tcPO2 by Condition Over Time

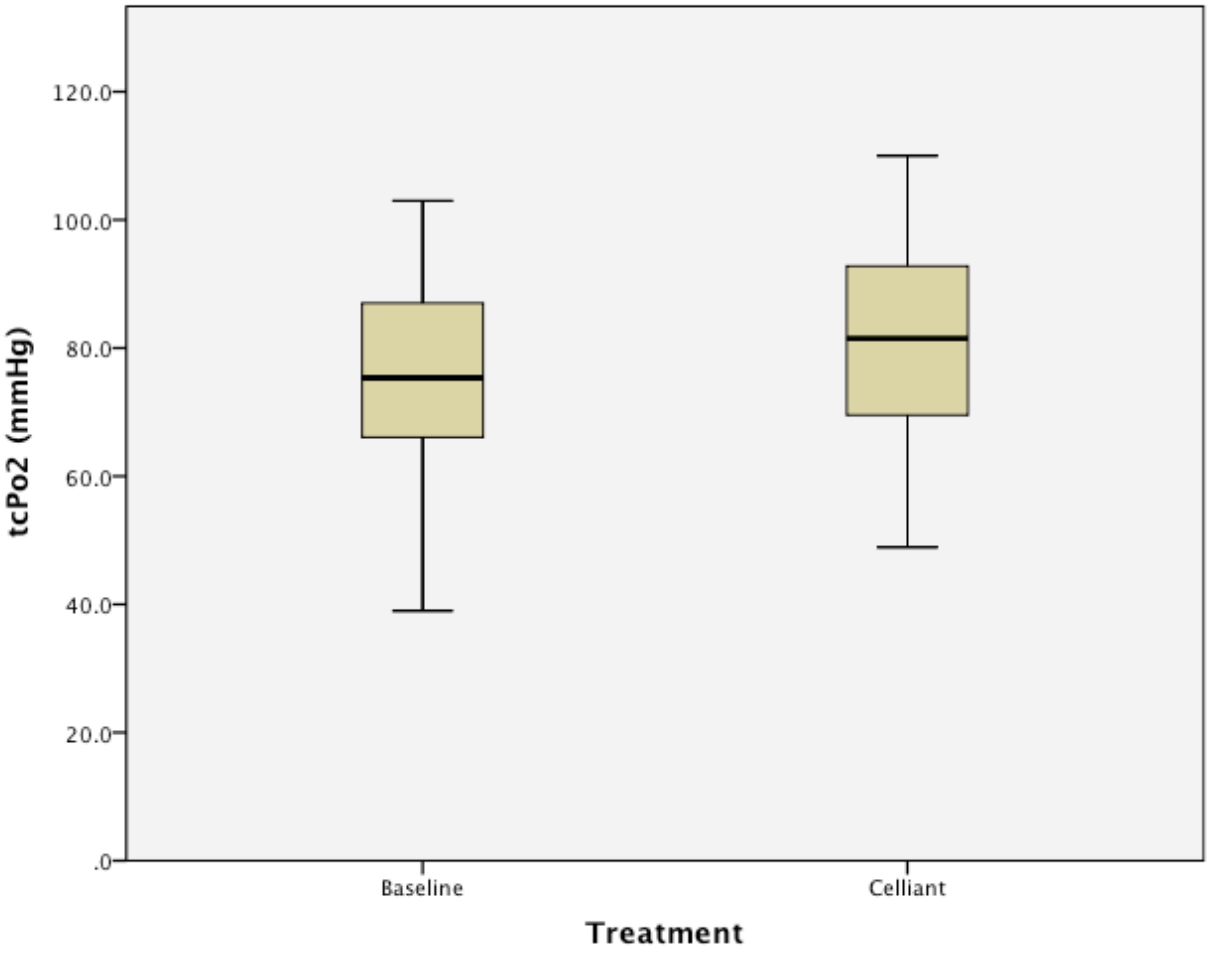


Error Bars: 95% CI

* At all time points, Celliant was statistically greater than Baseline. See Table 1 for p values.

A boxplot of the treatment means (Figure 3) is helpful to identify skewed data. The median is the line in the middle of the box; the upper edge of the box is the 75th percentile and the lower edge is the 25th percentile. The ends of the vertical bars or "whiskers" indicate the minimum and maximum data values. A datum outside the whiskers is an outlier, in this case there were none (McGill, Tukey, & Larsen, 1978). This graphic suggests that these data show good symmetry and are not skewed.

Figure 3. Distribution of tcPO2 values Between Baseline & Celliant



SUMMARY

Fifty-one subjects underwent separate ninety (90) minute testing periods where transcutaneous partial pressure of oxygen ($tcPO_2$), a well-accepted clinical measurement for tissue perfusion and oxygenation, was used as the primary endpoint. First, a Baseline was established. Then, a shirt made with Celliant® material was worn during a subsequent session. All measurements were taken at the bicep.

Transcutaneous oxygen tension proved to be a sensitive measure of efficacy, which resulted in the rejection of the null hypothesis. Thus, mean $tcPO_2$ values for the Celliant® garment were statistically greater than the Baseline at all time points (t= 10-min, 30-min & 90-min), as well as showed an over all treatment effect when mean $tcPO_2$ values were condensed across time. In all cases, the observed mean increases in $tcPO_2$ represented a seven percent (7%) increase in tissue oxygenation in the Celliant® condition when compared to Baseline.

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Multi-channel

$tcpO_2/pCO_2$ and Blood Perfusion monitor

System 5000 is unique in permitting up to four channels of $tcpO_2/pCO_2$ and laser Doppler in any combination.

The microvascular status of a leg is being monitored by a four channel laser Doppler and Transcutaneous Oxygen monitor

Blood perfusion measurements

with laser Doppler

The PeriFlux System 5000 is the fifth generation of the Perimed PeriFlux instrument range for microvascular assessment. It is a compact, easy to use, multi-functional system that can incorporate up to four Function Units. The combination of tcpO_2 / pCO_2 and laser Doppler Function Units provides more information about tissue perfusion, oxygenation and metabolic function than the two methods individually.

PF 5001 Main Unit with one LDPM Unit, one Temp Unit and two tcpO_2 Units.

Laser Doppler perfusion measurements

Low power laser light is transmitted to the tissue via a fibre optic probe. The returning light is processed and the relative number and velocity of the blood cells in the tissue are calculated and presented as **blood perfusion**. Laser Doppler has been extensively used for tissue evaluation on skin and most other organs (see Perimed Literature Reference List containing more than 1300 research articles).

The technique has recently grown in importance in the diagnosis and treatment of hypoxia and ischaemia-related tissue disorders. Valuable information is provided for:

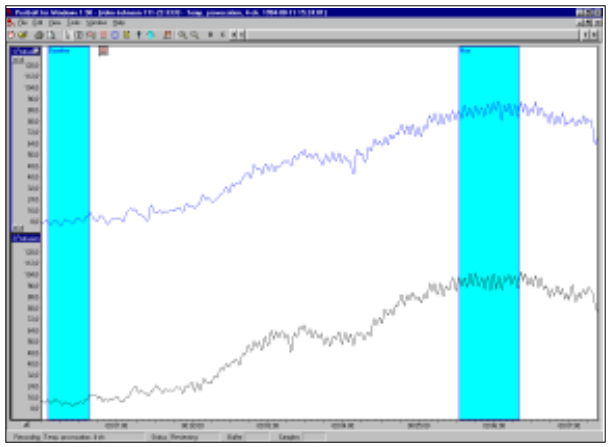
- Management of peripheral vascular disease
- Diabetes treatment
- Amputation level determination
- Monitoring therapy
- Plastic surgery (evaluation of flaps)
- Treatment of burns

Wound healing

An excellent technique to assess the wound healing process is provided by the combination of tcpO_2 / pCO_2 and laser Doppler. The laser Doppler measures perfusion while the tcpO_2 / pCO_2 measures oxygenation and metabolic function of the tissue.

Tissue perfusion capacity

To find the maximum perfusion capacity of a tissue, a combined laser Doppler and heat probe is used and the tissue is heated (e.g. 44°C). The perfusion change before and after the local heating is a measure of the tissue reserve capacity.



Wound healing assessment using a two channel laser Doppler and local heating of tissue to detect maximum dilatation.

Accurate and reliable

The unique linearization function prevents underestimation in well-perfused tissue. Two-point calibration (using automatic zeroing and a special motility standard) ensures that all probes will provide accurate and reliable results for the best patient care.

Simple to operate/Fast results

The probes are applied in seconds and results are obtained in minutes.

Oxygenation and tissue metabolism

with tcpO_2 / pCO_2

Transcutaneous monitoring of oxygen and carbon dioxide, originally developed for neonatal use, is now used in a number of different applications, including vascular and wound care, plastic surgery, hyperbaric medicine and orthopaedic surgery.

tcpO_2 / pCO_2 measurements

The technique is very easy to use and gives accurate, reliable measurements for tissue evaluation. It has now become a routine measurement in several clinical areas:

- Determination of peripheral vascular oxygenation.
- Quantification of the degree of peripheral vascular disease.
- Determination of the optimum level of amputation.
- Establishing the level of tissue hypoxia in venous disease.

Multichannel system

tcpO_2 measurements usually require at least two or three sites to provide a good picture. The more sites that are assessed, the better the oxygenation picture.

The PeriFlux 5000 now offers the capability of measuring up to four sites on a patient from one instrument. This has several advantages over using individual single channel monitors, for example:

- Greater portability.
- Saves space.
- Easier identification of measurement sites.
- Less risk of damage to electrodes and monitors.
- Patient data from multiple sites easily downloads to a PC for storage, editing and print out of reports.

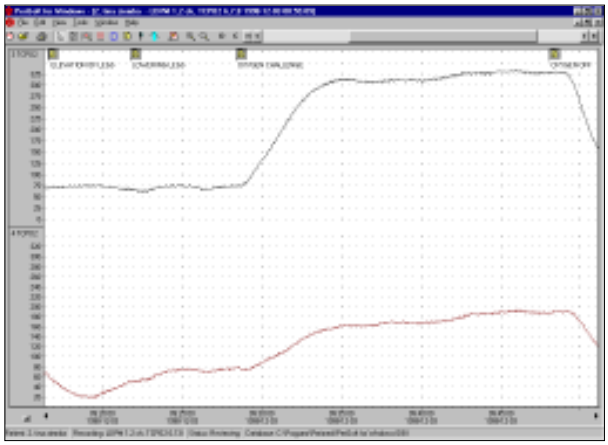
A four channel tcpO_2 system

Ease of use

The single point calibration is typically completed in less than three minutes, making it possible to save time and costs. Attachment of the electrode takes a few seconds using rings specially designed for patient safety and reliability of measurement.

Superior sensor technology

The multichannel monitor utilizes Radiometer electrodes – well known for superior durability and reliability. The electrodes are safe for use in 100% oxygen at pressures of up to 4 atm.



Recording of a two channel tcpO_2 system. Result of leg elevation and oxygen challenge. The upper trace shows the response from a healthy leg, while the lower trace shows a leg with vascular problems.

Specifications

PeriFlux 5001 Main Unit and 5002 Basic Main Unit

The **PeriFlux 5001 Main Unit** is provided with a solid state diode laser and can host up to four Function Units of different types. More channels are added using another Main Unit. The Main Unit has a digital output for connection to Perimed's PeriSoft software or a dedicated printer. It also has an analog output for connection to pen recorders or computer systems. Alternatively it can be used stand-alone, without external data acquisition systems.
The **PeriFlux 5002 Basic Main Unit** is equivalent to 5001, but is not provided with laser diode and can only host the Function Units PF 5020 Temp Unit and PF 5040 tcpO_2 / pCO_2 Unit.

Function Units

The Function Units are designed to meet specific needs in different applications. To optimize the system, the customer can choose the appropriate unit to meet particular requirements. Additional Function Units can easily be subsequently installed by the user.

PF 5010 LDPM Unit (Laser Doppler Perfusion Monitor)

For real-time microvascular perfusion measurements. The system uses the extensive PeriFlux System 4000 range of laser Doppler probes to allow measurements in virtually all tissues, invasive or non-invasive.

PF 5020 Temp Unit

For local heat provocation and/or temperature measurements. During a heat provocation the microvascular response is measured with the laser Doppler. This response indicates the maximum dilatation capacity of the tissue.

PF 5040 tcpO_2 / pCO_2 Unit

For measurement of transcutaneous oxygen and transcutaneous carbon dioxide. tcpO_2 / pCO_2 is measured from a combined electrode and tcpO_2 from a single electrode. Calibration of the O_2 electrode can be performed using atmospheric air. The CO_2 electrode requires the use of a calibration unit.

Perisoft for Windows (95/98/NT)

Dedicated software for data collection, storage, analysis and printing. (See pamphlet)

Local Printer

For hard-copy documentation a printer can be connected.

PeriFlux 5001 and 5002

Laser (only 5001)	Solid-state diode laser: 780 nm. Maximum power output at probe tip is 1 mW.
Classification	CE approved according to MDD 93/42/EEC, Electrical Safety Standard EN 60601-1, Class I EMC Standard EN 60601-1-2. Laser: EN 60825-1 Class 1(CFR 1040.10 Class I).

Output

Digital	RS-232 output to computer or directly to printer.
Analog	-10 to +10 V.
Mains	Selectable - 115 or 230 V. 50 or 60 Hz.
Power cons.	60 VA with four modules.
Dimensions	300 (W) x 320 (D) x 105 (H) mm.
Weight	8.6 kg with four modules.
Local Printer	DPU-3445, Thermal Printer, Seiko

Function Units

PF 5010	**LDPM Unit**
	For one laser Doppler perfusion probe per unit.
Outputs	Perfusion and TB (Total Backscattered light).
Classification	Type CF.
Display	3 digit LED display.

PF 5020	**Temp Unit**
	For one or two Thermostatic Probes/Probe Holders/Measurement Sensors per unit.
Output	Measured Temperature at Probe/Sensor.
Heating Range	26-44°C; increments of 1°C. Accuracy ±0.5°C.
Sensor Range	30-40°C. Accuracy ±0.3°C, Repeatability ±0.1°C. 0-30°C and 40-50°C. Accuracy ±0.5°C, Repeatability ±0.1°C.
Classification	Type BF.
Display	3 digit LED display.

PF 5040	**tcpO_2 / pCO_2 Unit**
	For measurement with one electrode per unit.
Output range	tcpO_2: 0-1999 mmHg (0-267 kPa). tcpCO_2: 0-200 mmHg (0-26.7 kPa).
Temp. settings	37-45°C in increments of 0.5°C.
Temp. accuracy	±0.1°C.
Classification	Type BF.
Display	3.5 digit LED display.

Accessories

E5280	Combination electrode tcpO_2/pCO_2 solid state.
Response time	Typical tcpO_2: 20 sec, tcpCO_2: 50 sec.
Stability	Typically better than 1 mmHg/h.
E5250	Electrode tcpO_2 solid state.
Response time	Typical 20 sec.
Stability	Typically better than 1 mmHg/h.
D280	Membraning kit for tcpO_2/pCO_2 electrode.
D826	Membraning kit for tcpO_2 electrode.
D282	Fixation kit for E5280/E5250 electrodes.
TCC3	Calibration Unit for tcpO_2/pCO_2.
TC 510	Calibration gas 20.9% O_2 and 5% CO_2.
TC 100	Extension cable 6m for electrode E5280/E5250.
PF 5840	Remote Panel for tcpO_2/pCO_2 including cable.
PF 5810	Remote Panel for LDPM and Heat incl. cable.

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Data subject to change without notice
tcpo2-2.p65 Part no: 44-00007-02 2000.05.11

CELLIANT™

ENHANCE YOUR LIFE.

SAMPLE

Style/Composition: 100% Celliant Socks

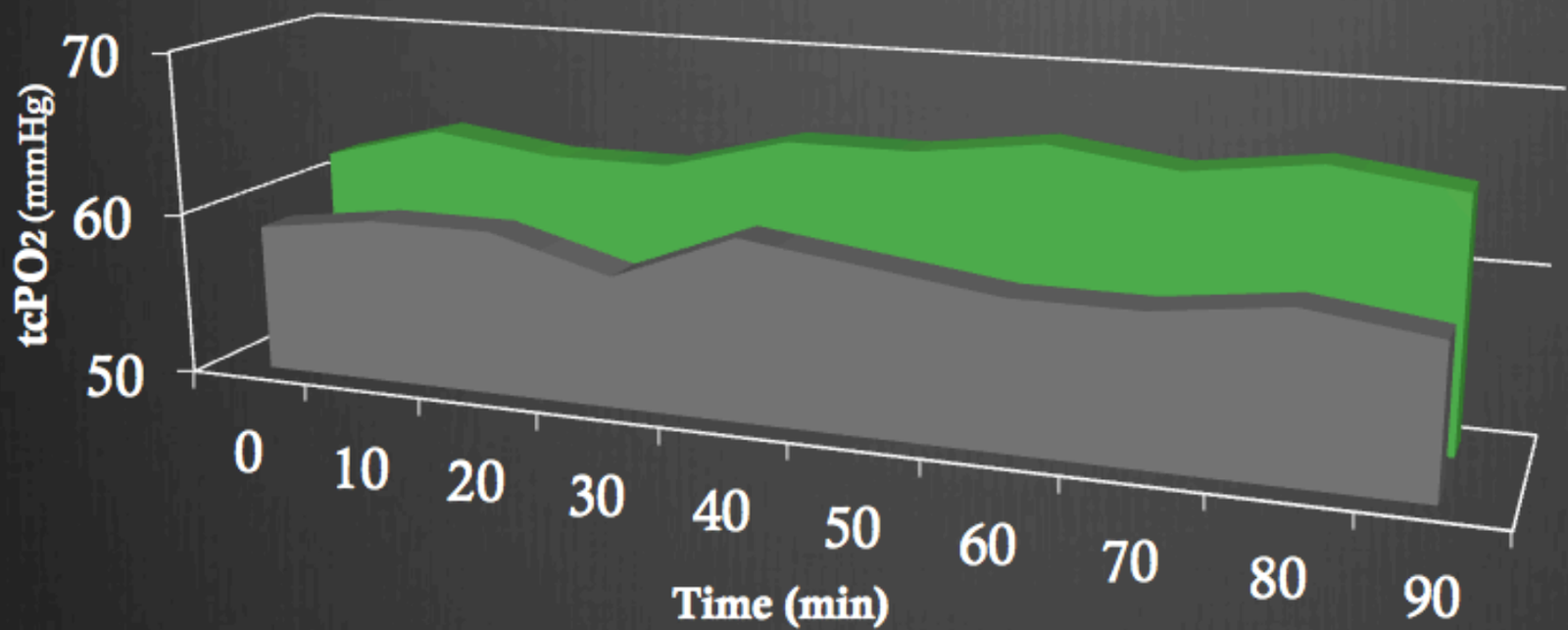
Vital Statistics and TCPO2 Data

Test Number:	42811
Date:	4/28/2011
Name:	Sean Hurdle
Age:	28
Sex:	Male
Probe Placements:	See below

Vitals	After 90 Minutes	
	Control	Celliant
Skin Temperature:	86	85
BP: Systolic/ Diastolic	160	161
	87	80
Heart Rate:	65	63
Grip Strength:	48	53

	TCPO2		
Minutes	Control	Celliant	% Change +/-
Baseline	38	44	15.79%
10	38	46	21.05%
20	39	48	23.08%
30	39	45	15.38%
40	38	48	26.32%
50	39	46	17.95%
60	37	46	24.32%
70	39	46	17.95%
80	37	48	29.73%
90	37	47	27.03%
		Average +/-	21.86%

The Evolution of Performance Technology



CELLIANT®

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Control

Celliant ®

Time	0	10	20	30	40	50	60	70	80	90
Control	59	60	60	58	61	60	59	59	60	59
Celliant ®	62	64	63	63	65	65	66	65	66	65

Maximum Increase

12%

12/16/11
SAMPLE
Style: SAMPLE
Comp: 44.9% Celliant 41.5% Polyester
13.6% Spandex
Test #1 of #1
#SAMPLE
Age: SAMPLE
Sex: SAMPLE
Room Temp: 23.3 °C

Control Vitals

Skin Temperature °C: 33.3

BP: Systolic/ Diastolic: 111/60

Heart Rate: 68

Celliant Vitals

Skin Temperature °C: 33.3

BP: Systolic/ Diastolic: 121/61

Heart Rate: 65

Celliant Effect

Time:	Celliant:
30 Min	**9%**
60 Min	**12%**
90 Min	**10%**

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