Universitätsklinikum
Jena

Klinik für Hautkrankheiten

Qualitäts-zertifiziert nach DIN EN ISO 9001:2008

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29. Februar 2012

study report:

In vitro evaluation of the anti-oxidative potential of textile sample "SeaCell® pure" (# 12 02 010)

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1. Quality certificate

Zertifikat

QUALITÄTSMANAGEMENTSYSTEM – DIN EN ISO 9001: 2008

Hiermit wird bestätigt, dass das

Universitätsklinikum Jena
Klinik für Dermatologie und
dermatologische Allergologie
Erfurter Straße 35
07740 Jena
Deutschland

Inhaber des Zertifikates Nr. **FS 519135/5409D**

ein Qualitätsmanagementsystem gemäß **DIN EN ISO 9001:2008** *für den folgenden Geltungsbereich anwendet:*

Dermatologie, Allergologie, Berufsdermatologie, Andrologie, Dermato-Histologie, Dermato-Onkologie, Hautphysiologie/Skin Study Center, Forschungslabor, Diagnostisches Labor, Operative Dermatologie, Laser, Photodermatologie, Proktologie, Phlebologie, Wundheilung

Für und im Namen von BSI:

Geschäftsführung, BSI Management Systems (Deutschland)

Ursprünglich zertifiziert: **12.12.2003** Letzte Ausgabe: **06.10.2009** Ablaufdatum: **16.10.2012**

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QMS/EMS-TGA-ZM-08-82

Dieses Zertifikat wurde elektronisch erstellt und bleibt Eigentum der BSI und ist an die Vertragsbedingungen gebunden.
Ein elektronisches Zertifikat kann online beglaubigt werden.
Kopien können auf www.bsigroup.de/de/Audit-und-Zertifizierung/138880/ oder per Telefon +49 (0) 6181 99370 validiert werden.

Die British Standards Institution ist eingetragen in die Royal Charter.
BSI Management Systems und Umweltgutachter Deutschland GmbH, Dörnigheimer Straße 2a, 63452 Hanau, Deutschland.

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2. General information

2.1 Test items

textile sample “SeaCell® pure” (# 12 02 010)

2.2 Reference items

reference sample (“SeaCell® pure”)

2.3 Sponsor

smartfiber AG
Breitscheidstraße 154
D-07407 Rudolstadt
Germany
Person responsible: Anita Varga

2.4 Test facility

Klinik für Hautkrankheiten
Universitätsklinikum Jena
Erfurter Straße 35
D-07740 Jena
Germany
Study director: PD Dr. Uta-Christina Hipler

2.5 Operating schedule

Start of experiments: 10.02.2012
End of experiments: 24.02.2012
Date of final report: 29.02.2012

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3. GLP and quality assurance statement

I assure that the Test facility complies with the Principles of Good Laboratory Practice. Appropriate and technically valid Standard Operating Procedures are established for the described tests. The Test facility is certified according to DIN EN ISO 9001:2008.

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29.02.12

Date

Study director: PD Dr. Uta-Christina Hipler

4. Summary

The purpose of this study was to investigate the antioxidative capacity against free radicals such as ROS (reactive oxygen species) and RNS (reactive nitrogen species) *in vitro*.
The textile sample "SeaCell® pure" (# 12 02 010) exhibited a significant antioxidative capacity *in vitro*. It could be shown that it is equally effective against ROS and RNS.

5. Background

The skin is the major interface between body and environment. It is the most versatile human organ and plays a key role in protecting the body against environmental influences and participates in the regulation of homeostasis, metabolic processes as well as immunological reactions. Oxidative stress by free radicals accelerates skin aging and has been implicated in dermatological diseases such as atopic dermatitis [Sezer et al. 2007, Briganti & Picardo 2003]. UV light induces the generation of free radicals in the cells; hence, the application of topical antioxidants has been recommended [Masaki 2010, Maela-Azulay & Bagatin 2009]. Textiles are the tissues with the longest contact to the human skin. In the clinically and cosmetically field they are used manifold; one scope of application is for instance the functionalization of fabrics with antioxidants.

The antioxidant capacity (AOC) of soluble substances and other materials, e.g. fabrics, can be monitored and quantified using *in vitro* tests. The several methods are based on different reaction mechanisms and employ various radicals and substrates. Peroxyl radicals ($ROO^{\bullet}$) are the most often used radicals for *in vitro* procedures as they present the key radical for auto oxidation of lipids [Ou et al. 2001]. The ROS (reactive oxygen species) test determines the inhibition of the Pholasin® oxidation by superoxide anions and other oxygen radicals. In contrast, the RNS (reactive nitrogen species) test measures the efficacy of an antioxidant to decrease the Pholasin® oxidation by peroxynitrite ($ONOO^-$). Pholasin® is a photo protein isolated from the mollusc *Pholas dactylus,* which emits light in the presence of certain oxidants (chemiluminescence). Previously these tests has been successfully used to determine the antioxidant capacity of wound dressings [Wiegand et al. 2009, Wiegand et al. 2006, Schönfelder et al. 2005] and textiles [Hipler & Wiegand 2011, Wiegand et al. 2010, Fluhr et al. 2010].

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6. Description of materials and test methods

6.1 Sample preparation

Textile samples were cut using 8 mm and 5 mm punch biopsies (Stiefel Laboratorium GmbH, Germany) corresponding to 0.5 cm^2 and 0.25 cm^2, respectively, and transferred to white 96-well plates (greiner bio-one, Germany).

6.1 Determination of the antioxidant capacity against ROS

The capability of the wound dressings to scavenge free radicals such as ROS (reactive oxygen species) was assessed using the chemiluminescent ABEL® Antioxidant Test Kits specific for superoxide anion and other radicals containing Pholasin® (Knight Scientific Limited, U.K.).

To each sample the respective assay solutions were added. In brief, 25 µL assay buffer (Lot. GA248A B2 100210), 50 µL Pholasin® solution (Lot. AA170A B1 110428) as well as 100 µL solution A (Lot. JA510A A1 110202) were added. Then 25 µL of solution B (Lot. KA610A B3 090930) were injected to each well immediately before measurement. A control without sample was run with each assay. The measurement of luminescence was carried out using the LUMIstar Galaxy plate reader (BMG Labtech GmbH, Germany).

The antioxidant capacity of a sample is expressed as percent reduction of peak luminescence as follows:

$$\% \text{ inhibition} = \frac{[(\text{peak-control}) - (\text{peak-sample})] \times 100}{(\text{peak-control})}$$

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6.1 Determination of the antioxidant capacity against RNS

The capability of the wound dressings to scavenge free radicals such as RNS (reactive nitrogen species) was assessed using the chemiluminescent ABEL® Antioxidant Test Kits specific for peroxynitrite anion containing Pholasin® (Knight Scientific Limited, U.K.).
To each sample the respective assay solutions were added. In brief, 100 µL assay buffer (Lot. TA505 B2 100129) as well as 50 µL Pholasin® solution (Lot. AA170A A2 110203) were added. Then 50 µL of SIN-1 solution (Lot. UB711B B3 110201) were injected to each well prior to the measurement. A control without sample was run with each assay. The measurement of luminescence was carried out using the LUMIstar Galaxy plate reader (BMG Labtech GmbH, Germany).
The antioxidant capacity of a sample is expressed as percent reduction of peak luminescence as follows:

$$\% \text{ inhibition} = \frac{[(\text{peak-control}) - (\text{peak-sample})] \times 100}{(\text{peak-control})}$$

6.4 Statistics

Experiments were performed in duplicate and measurements were performed in triplicate. All values are expressed as means ± SD (standard deviation). One-way analysis of variance was carried out to determine statistical significances (Microsoft® Excel 2000). Differences are considered statistically significant at a level of $p < 0.05$. Asterisks indicate significant deviations from the control ($* p < 0.05$; $** p < 0.01$; $*** p < 0.001$).

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7. Deviations from the study protocol

There were no deviations from the study protocol.

8. Archiving

The following records will be stored in the archives of the Klinik für Hautkrankheiten, Universitätsklinikum Jena according to the GLP regulations:
A copy of the final report, the study plan and a documentation of all raw data generated during the conduct of the study will be stored for at least 4 years after completion of the study.
Unused test items and reference items are stored for at least 12 month after completion of the study.
Materials and samples that are unstable may be disposed of before that time and without sponsor's prior consent.
Records and reports of the maintenance and calibration of apparatus, validation documentation for computerized systems and the historical file of all Standard Operating Procedures (SOPs) is stored in accordance with the appropriate authorities.

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9. Results and discussion

Textile sample "SeaCell® pure" (# 12 02 010) exhibited a significant, concentration-dependent capacity to inhibit the formation of free reactive oxygen species (Figure 1). Furthermore, textile sample "SeaCell® pure" (# 12 02 010) was able to reduce the formation of reactive nitrogen species (Figure 2). A similar antioxidant capacity for ROS and RNS could be observed *in vitro*. No significant difference between sample "SeaCell® pure" (# 12 02 010) and the reference sample was found.

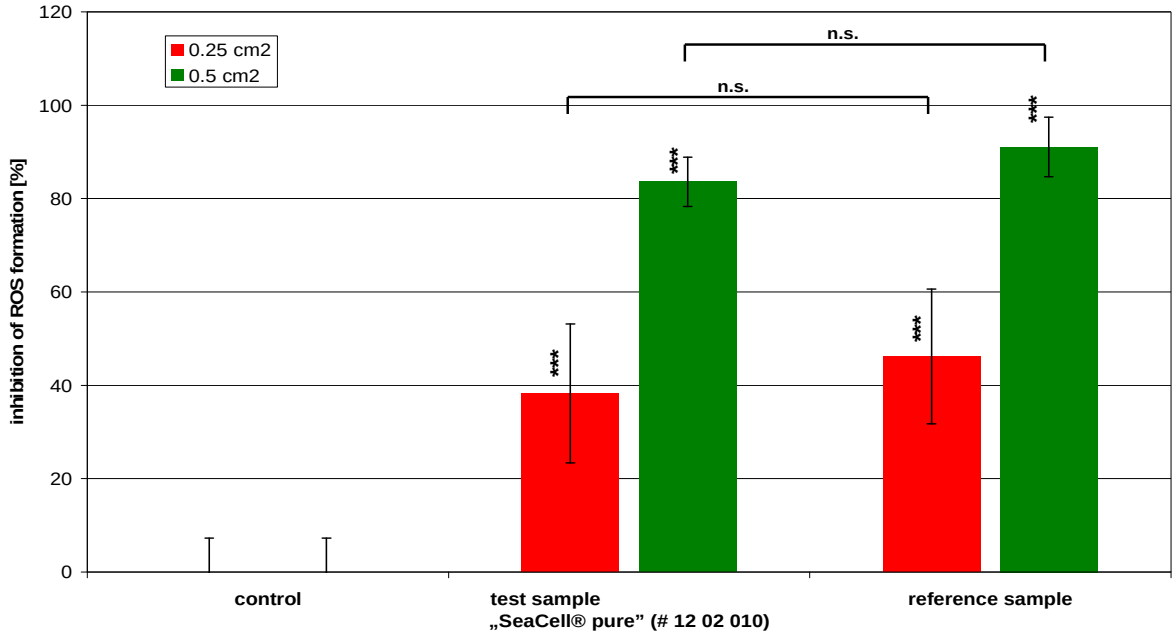

Figure 1: Inhibition of ROS formation (for data see table 1 in the appendix)

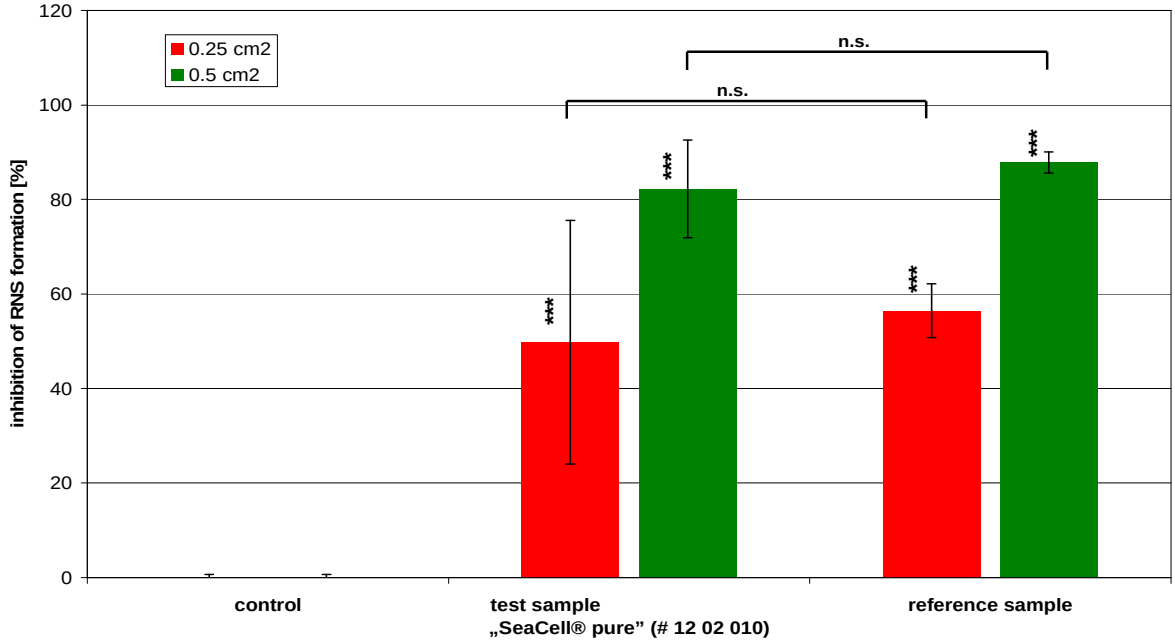

Figure 2: Inhibition of RNS formation (for data see table 2 in the appendix)

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10. Appendix

10.1 Abbreviations

cm^2	square centimeters
M	molar
mL	milliliters
µL	microliters
mm	millimeters
mM	millimolar
n.s.	not significant
RNS	reactive nitrogen species
ROS	reactive oxygen species
SD	standard deviation

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10.2 Tables and Figures

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10.3 References

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10.4 Measurement data

Table 1: Reduction of ROS formation.

	sample size	0.25 cm^2		0.5 cm^2	
		ROS formation	ROS inhibition	ROS formation	ROS inhibition
control	**[%]**	91.97	8.03	91.97	8.03
		100.37	-0.37	100.37	-0.37
		107.65	-7.65	107.65	-7.65
		108.94	-8.94	108.94	-8.94
		98.85	1.15	98.85	1.15
		92.21	7.79	92.21	7.79
	mean	**100.0**	**0.0**	**100.0**	**0.0**
	SD	**7.3**	**7.3**	**7.3**	**7.3**
test sample "SeaCell® pure" (# 12 02 010)	**[%]**	60.9	39.07	9.8	90.24
		87.2	12.81	20.1	79.95
		70.7	29.29	10.5	89.50
		50.5	49.52	20.9	79.13
		50.5	49.46	21.5	78.46
		50.60	49.40	15.84	84.16
	mean	**61.7**	**38.3**	**16.4**	**83.6**
	SD	**14.9**	**14.9**	**5.3**	**5.3**
	p-value (control)		0.0002		0.0001
reference sample	**[%]**	68.5	31.53	19.7	80.27
		68.8	31.24	12.1	87.87
		63.5	36.55	10.2	89.78
		41.8	58.24	3.6	96.39
		40.3	59.70	4.0	95.99
		40.25	59.75	3.99	96.01
	mean	**53.8**	**46.2**	**8.9**	**91.1**
	SD	**14.4**	**14.4**	**6.4**	**6.4**
	p-value (control)		0.0001		0.0001

Table 2: Reduction of RNS formation.

	sample size	0.25 cm^2		0.5 cm^2	
		ROS formation	ROS inhibition	ROS formation	ROS inhibition
control	**[%]**	100.51	-0.51	100.51	-0.51
		99.31	0.69	99.31	0.69
		100.18	-0.18	100.18	-0.18
		99.26	0.74	99.26	0.74
		100.89	-0.89	100.89	-0.89
		99.84	0.16	99.84	0.16
	mean	**100.0**	**0.0**	**100.0**	**0.0**
	SD	**0.7**	**0.7**	**0.7**	**0.7**
test sample "SeaCell® pure" (# 12 02 010)	**[%]**	70.5	29.47	9.1	90.86
		53.7	46.33	12.9	87.06
		86.3	13.71	34.7	65.32
		19.9	80.08	17.9	82.08
		46.1	53.88	7.6	92.44
		24.5	75.49	24.52	75.48
	mean	**50.2**	**49.8**	**17.8**	**82.2**
	SD	**25.8**	**25.8**	**10.3**	**10.3**
	p-value (control)		0.0008		0.0001
reference sample	**[%]**	38.3	61.72	10.4	89.63
		37.2	62.76	12.9	87.07
		41.0	59.02	14.9	85.08
		50.8	49.22	14.4	85.57
		44.4	55.61	9.4	90.62
		49.5	50.52	10.92	89.08
	mean	**43.5**	**56.5**	**12.2**	**87.8**
	SD	**5.7**	**5.7**	**2.3**	**2.3**
	p-value (control)		0.0001		0.0001