



**Universitätsklinikum
Jena**

Klinik für Hautkrankheiten

Qualitäts-zertifiziert nach DIN EN ISO 9001:2008

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28. Juli 2014

study report:

***In vitro* evaluation of the
anti-oxidative potential of the
textile sample “SeaCell[®]” (# 14 07 085)
compared to cotton (# 14 07 086)**

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1. Quality certificate



Certificate of Registration

QUALITY MANAGEMENT SYSTEM – DIN EN ISO 9001: 2008

This is to certify that

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Klinik für Hautkrankheiten**
Erfurter Straße 35
07740 Jena
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Holds Certificate No: FS 519135/5409D

and operates a Quality Management System which complies with the requirements of DIN EN ISO 9001:2008 for the following scope:

Dermatology, Allergology, Occupational Dermatology, Andrology,
Dermatohistology, Dermatocarcinology, Skin Physiology/Skin Study Center,
Research Laboratory, Diagnostic Laboratory, Dermatological Surgery,
Laser, Photodermatology, Proctology, Phlebology, Wound Healing,
Trichology, Venerology, Pediatric Dermatology

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2. General information

2.1 Test items

textile sample "SeaCell[®]" (# 14 07 085)

2.2 Reference items

reference sample cotton (#14 07 086)

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: 2014-07-17

End of experiments: 2014-07-24

Date of final report: 2014-07-28

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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.

PD Dr. U.-C. Hipler

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28.07.2014

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[®]" (# 14 07 085) exhibited a highly significant antioxidative capacity *in vitro*. It could be shown that it is equally effective against ROS and RNS. The antioxidative capacity observed was distinctly higher than that of the reference sample cotton (# 17 07 086).

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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[•]) 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].



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 cm² and 0.25 cm², 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. GA251A B1 120809), 50 µL Pholasin[®] solution (Lot. AA170A B1 110428) as well as 100 µL solution A (Lot. JA511A A1 130422) were added. Then 25 µL of solution B (Lot. KA611A B3 110912) 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:

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



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 peroxyxynitrite anion containing Pholasin[®] (Knight Scientific Limited, U.K.).

To each sample the respective assay solutions were added. In brief, 100 µL assay buffer (Lot. TA 508A B2 120416) as well as 50 µL Pholasin[®] solution (Lot. AA 170A A2 130520) were added. Then 50 µL of SIN-1 solution (Lot. UB712B 120330) 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 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).



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.



9. Results and discussion

Textile sample “SeaCell®” (# 14 07 085) exhibited a significant, concentration-dependent capacity to inhibit the formation of free reactive oxygen species (Figure 1). Furthermore, textile sample “SeaCell®” (# 14 07 085) was able to reduce the formation of reactive nitrogen species (Figure 2). The reference sample cotton (# 17 07 086) achieved a significant antioxidative effect against ROS but only a slight inhibition of RNS. In accordance, a significant difference between the textile sample “SeaCell®” (# 14 07 085) and the reference sample was observed ($p < 0.001$).

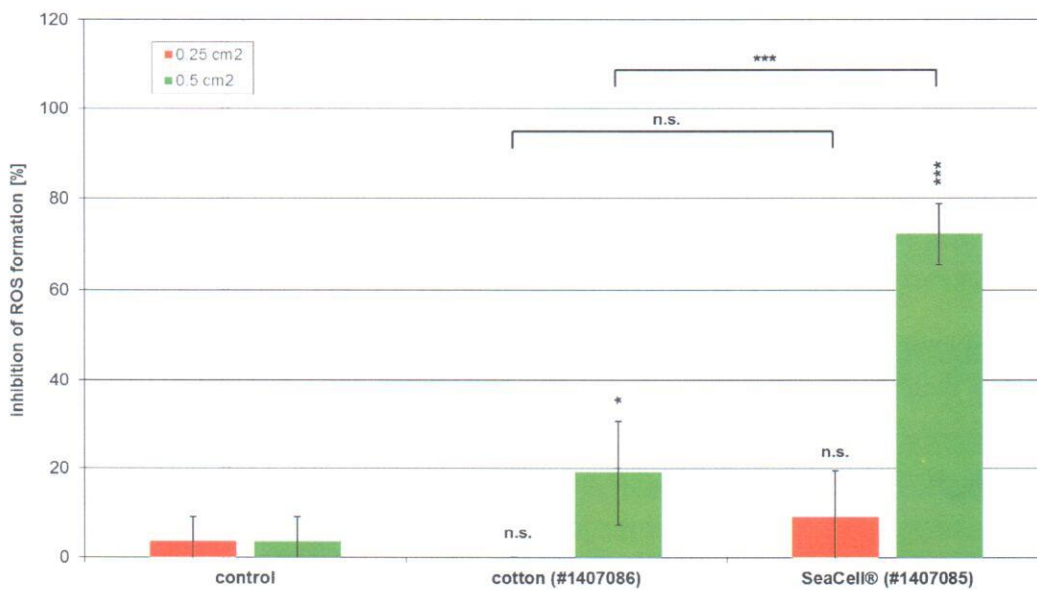


Figure 1: Inhibition of ROS formation (for data see table 1 in the appendix)

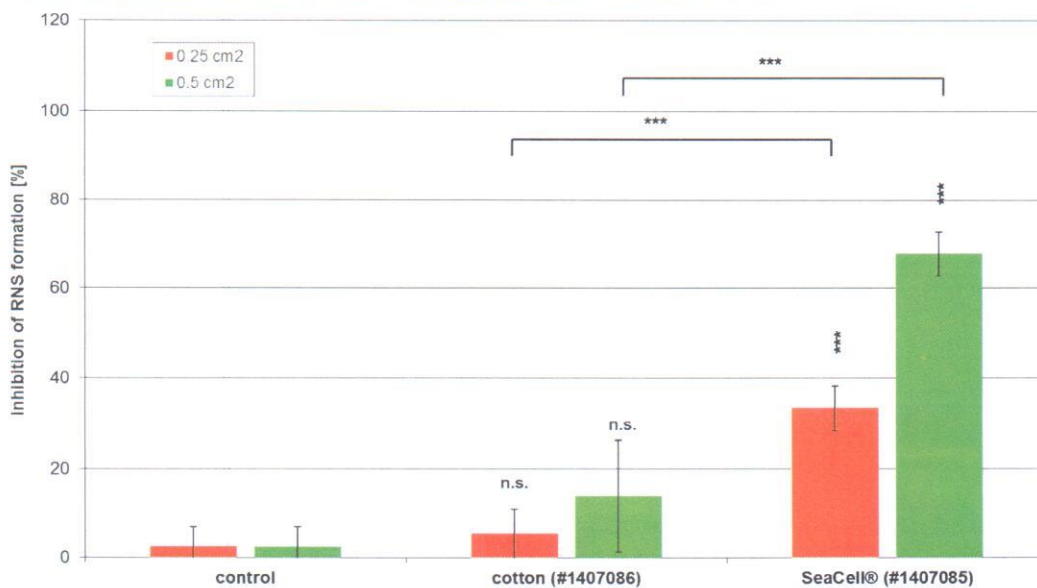


Figure 2: Inhibition of RNS formation (for data see table 2 in the appendix)

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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



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
control	[%]	0.00	0.00
		0.00	0.00
		6.43	6.43
		0.00	0.00
		0.00	0.00
		13.89	13.89
	mean	3.4	3.4
SD	5.8	5.8	
reference sample cotton (# 14 07 086)	[%]	0.00	5.54
		0.00	28.72
		0.00	15.66
		0.00	15.53
		0.00	11.21
		0.00	36.75
	mean	0.0	18.9
SD	0.0	11.6	
p-value (control)	0.1798	0.0150	
textile sample "SeaCell" [®] (# 14 07 085)	[%]	23.54	67.29
		0.00	69.94
		10.63	62.52
		0.00	76.58
		19.16	78.11
		0.00	78.73
	mean	8.9	72.2
SD	10.6	6.6	
p-value (control)	0.2896	0.0001	

Table 2: Reduction of RNS formation.

	sample size	0.25 cm ²	0.5 cm ²
control	[%]	0.00	0.00
		0.00	0.00
		12.71	12.71
		1.36	1.36
		0.00	0.00
		0.00	0.00
	mean	2.3	2.3
SD	4.7	4.7	
reference sample cotton (# 14 07 086)	[%]	11.47	3.92
		0.00	35.01
		7.34	10.91
		0.00	2.53
		0.00	26.70
		13.09	4.04
	mean	5.3	13.9
SD	5.6	12.5	
p-value (control)	0.3824	0.0834	
textile sample "SeaCell" [®] (# 14 07 085)	[%]	30.11	67.22
		37.43	72.75
		40.72	61.34
		34.40	68.34
		26.06	74.52
		31.31	62.16
	mean	33.3	67.7
SD	4.8	4.9	
p-value (control)	0.0001	0.0001	

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