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In vitro evaluation of the anti-oxidative potential of the textile sample "SeaCell®"

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1. 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. UV light induces the generation of free radicals in the cells; hence, the application of topical antioxidants has been recommended. 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 ($ROO^{\cdot}$) are the most often used radicals for *in vitro* procedures as they present the key radical for auto oxidation of lipids. 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 *Pho/as dactylus,* which emits light in the presence of certain oxidants (chemiluminescence). Previously these tests have been successfully used to determine the antioxidant capacity of wound dressings and textiles.

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2. Description of materials and test methods

2.1 Sample preparation

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

2.2 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, 50 µL Pholasin® solution as well as 100 µL solution A were added. Then 25 µL of solution B 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 BioTek plate reader (BioTek Instruments, Inc, United States).

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

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

2.3 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 as well as 50 µL Pholasin® solution were added. Then 50 µL of SIN-1 solution 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 BioTek plate reader (BioTek Instruments, Inc, United States).

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

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

2.4 Statistics

Experiments were performed in duplicate and measurements were performed in triplicate. All values are expressed as means ± SD (standard deviation). One-way ANOVA analysis of variance was carried out to determine statistical significances (Microsoft® Excel 2007). Differences are considered statistically significant at a level of $p < 0.05$. Asterisks indicate significant deviations from the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Description	BL ID
Specimen #1508091: Stnadard cotton fabric, black	1508G15-1
Specimen #1508089: Coton/SeaCell-Lyocell fabric blend, black	1508G15-2
Specimen #1508090: Cotton/SeaCell-Modal fabric-blend, black	1508G15-3

3. Results and discussion

Textile samples "SeaCell®" (1508G15-1, 1508G15-2 and 1508G15-3) exhibited significant, concentration-dependent capacity to inhibit the formation of free reactive oxygen species (Figure 1). Furthermore, textile samples "SeaCell®" (1508G15-1, 1508G15-2 and 1508G15-3) were able to reduce the formation of reactive nitrogen species (Figure 2). In accordance, significant difference between the textile samples "SeaCell®" (1508G15-1, 1508G15-2 and 1508G15-3) and the control group were observed ($p < 0.001$).

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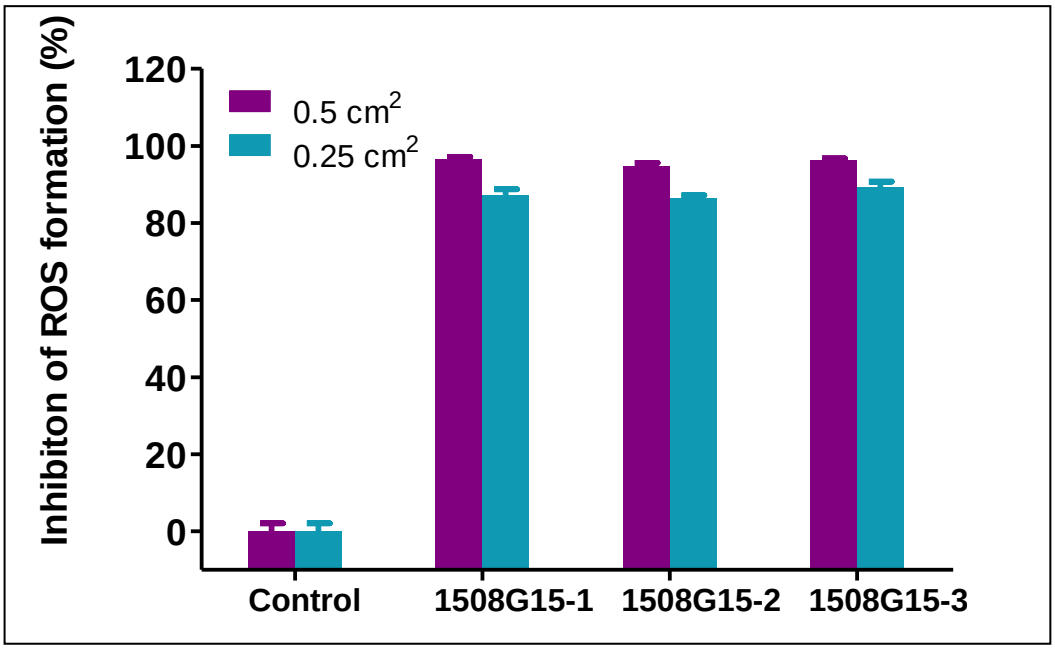

Figure 1. Inhibition of ROS formation (for data see table 1)

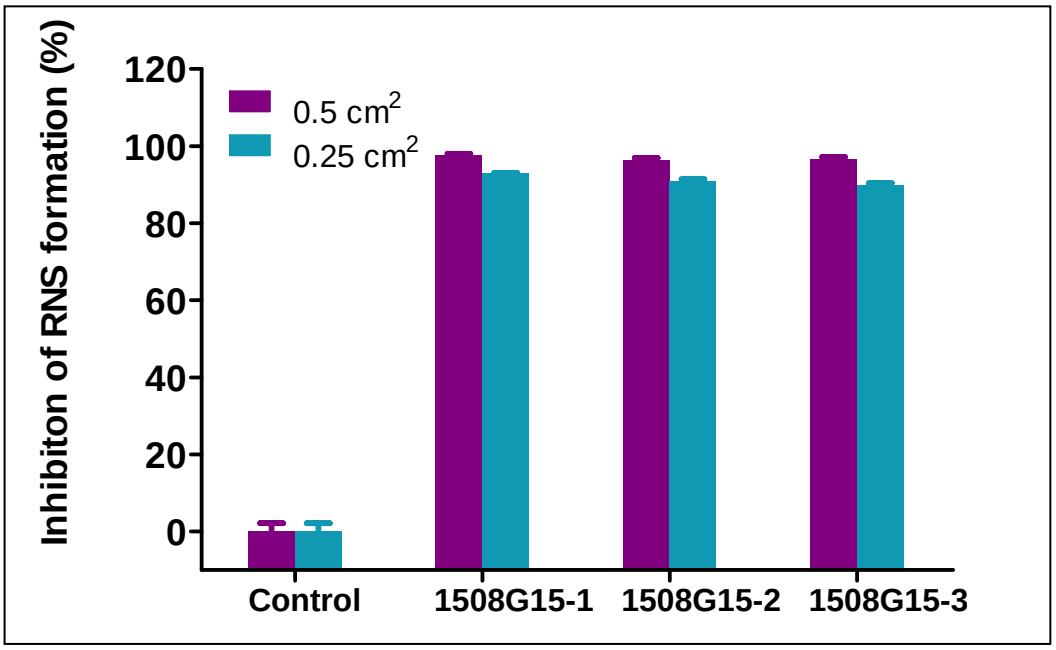

Figure 2. Inhibition of RNS formation (for data see table 2)

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4. Processed data

Table 1. Reduction of ROS formation.

	Sample size	0.25 cm^2	0.5 cm^2
Control	**%**	-2.19	-2.19
		-1.65	-1.65
		-2.27	-2.27
		-6.14	-6.14
		7.78	7.78
		4.46	4.46
	Mean	**0.00**	**0.00**
	SD	**5.11**	**5.11**
1508G15-1	**%**	91.82	96.10
		91.49	97.21
		86.13	97.64
		84.42	98.17
		86.99	93.01
		82.60	96.29
	Mean	**87.24**	**96.40**
	SD	**3.74**	**1.84**
	P value (vs. Control)	**< 0.001**	**< 0.001**
1508G15-2	**%**	86.44	95.81
		84.70	97.20
		86.40	91.61
		89.09	95.76
		84.19	91.58
		87.96	95.70
	Mean	**86.46**	**94.61**
	SD	**1.87**	**2.40**
	P value (vs. Control)	**< 0.001**	**< 0.001**
1508G15-3	**%**	90.31	98.64
		90.53	95.97
		94.93	95.71
		83.62	96.27
		88.47	96.12
		87.49	94.56
	Mean	**89.23**	**96.21**
	SD	**3.75**	**1.34**
	P value (vs. Control)	**< 0.001**	**< 0.001**

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Table 2. Reduction of RNS formation.

	Sample size	0.25 cm^2	0.5 cm^2
Control	%	-3.38	-3.38
		-3.70	-3.70
		0.32	0.32
		-0.21	-0.21
		10.45	10.45
		-3.49	-3.49
	Mean	**0.00**	**0.00**
	SD	**5.41**	**5.41**
1508G15-1	%	93.13	98.15
		93.03	95.96
		92.70	98.29
		92.02	98.47
		93.73	97.58
		92.30	97.19
	Mean	**92.82**	**97.61**
	SD	**0.62**	**0.94**
	P value (vs. Control)	**< 0.001**	**< 0.001**
1508G15-2	%	92.10	97.57
		89.48	96.21
		90.95	95.91
		92.74	98.06
		90.46	93.59
		89.69	96.41
	Mean	**90.90**	**96.29**
	SD	**1.30**	**1.57**
	P value (vs. Control)	**< 0.001**	**< 0.001**
1508G15-3	%	89.30	95.40
		91.37	98.44
		88.53	95.60
		89.55	97.76
		88.65	94.96
		91.78	97.17
	Mean	**89.86**	**96.55**
	SD	**1.39**	**1.43**
	P value (vs. Control)	**< 0.001**	**< 0.001**