Final report on testing the antimicrobial activity of the product OKCEL

As part of the Work Contract the antimicrobial efficiency of OKCEL products (topical absorbable haemostats, manufactured by Synthesia, a.s) was tested on the selected microorganisms (list of tested bacterial strains is stated in the attached Table No. 1). The testing was performed using both dilution and diffusion methods.
Testing was conducted at two facilities of the Department of Biological and Biochemical Sciences, University of Pardubice, namely the workplace of Food Microbiology (responsible person doc. Ing. Jarmila Vytřasová, Ph.D., Ing. Petra Moťková, Ph.D. and Drahomíra Hofmanová) and workplace of Clinical Microbiology (responsible person doc. MVDr. Jaroslava Mazurová, Ph.D., Mgr. Rudolf Kukla and RNDr. Petra Mosio, Ph.D.)

Working Procedure
At first the bacterial suspensions were prepared (density of cells was adjusted to 10^8 cfu/ml according to 0.5 degrees of Mc Farland's turbidity scale). A suspension of each bacteria in saline solution was always prepared from a fresh 24 hour culture of the bacteria. Decimal dilution series were prepared by diluting the cells down to a density of 10^3 cfu/ml. Control monitoring of the actual number of cells was carried out from the dilution of 10^3 cfu/ml by spreading the volume of 100 ml by L- stick on the surface of the soil according to needs of each bacteria strain (Table No. 1) and incubated under optimal temperature for the optimal time. All the tests were always performed in duplicate.

Dilution (suspension) method
1 g sample of tested OKCEL was placed into a wide plastic tube, containing 2 ml of bacterial suspension (density of 10^8 cfu/ml) and 18 ml of appropriate nutrient medium. After 6, 24 and 48 hours, the surface of the appropriate cultivation media was inoculated by 100 ml of the solution and spread by L-stick. Incubations were carried out under optimal conditions for each microorganism. The grown colonies were counted after incubation and the number of logarithmic reduction of inhibited growth of each bacterial strain under exact time period of interaction with OKCEL was found out (Table 2).
The blank tests (without the tested sample) were simultaneously executed with all tested microbes. Further procedure was the same as described above.

Diffusion method
Fresh 24 hour culture of the relevant microbe was spread on the surface of the Petri dish with an appropriate nutrient medium so as to uniformly cover the entire surface of the dish. After the surface was dry, then the tested sample of OKCEL was aseptically placed and pressed against the surface of the soil. Cultivation was carried out from 24 to 48 hours. Then the inhibition zone were measured (Table 3). After removal of the tested sample, the smear of the cultivation media was carried out and again inoculated on the surface of new cultivation media and incubated. This confirmed whether there was a complete inhibition of microbial growth.

Results

Table 1: List of bacteria, nutrient media and the optimum time and temperature of incubation

Microorganisms		Nutrient Media	Temperature/Time of incubation
CCM 4223	*Staphylococcus aureus*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 4418	*Staphylococcus epidermidis*	Nutrient agar No. 2	37 °C/ 24-48 hours
Clinical isolate	*Streptococcus pyogenes group A*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 6187	*Streptococcus agalactiae group B*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 4046	*Streptococcus salivarius*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
Clinical isolate	*Branhamella catarrhalis (Moraxella)*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 3954	*Escherichia coli*	Nutrient agar No. 2	37 °C/ 24-48 hours
Clinical isolate	*Klebsiella pneumoniae*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 4420	*Salmonella enteritidis*	Nutrient agar č. 2	37 °C/ 24-48 hours
CCM 303	*Serratia marcescens*	Nutrient agar No. 2	30 °C/ 24-48 hours
Clinical isolate	*Methicillin-resistant Staphylococcus aureus (MRSA)*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
Clinical isolate	*Penicillin-resistant Streptococcus pneumoniae (PRSP)*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours (10% CO_2)
Clinical isolate	*Vankomycin-resistant Enterococcus (VRE)*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
Clinical isolate	*Methicilin-resistant Staphylococcus epidermidis (MRSE)*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 1799	*Proteus spp.*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 1729	*Corynebacterium xerosis*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
Clinical isolate	*Mycobacterium smegmatis*	Lowenstein-Jensen's media	37 °C/ 72 hours
CCM 4435	*Clostridium perfringens*	blood agar with 7% ram blood	37 °C/ 24-48 hours, anaerobically
CCM 4508	*Bacteroides fragilis*	Wilkins-Chalgren's agar with 7% ram's blood	37 °C/ 120 hours, anaerobically

CCM 4224	*Enterococcus faecalis*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 1903	*Enterobacter cloacae*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 3955	*Pseudomonas aeruginosa*	Nutrient agar No. 2	37 °C/ 24-48 hours
CCM 2660	*Pseudomonas stutzeri*	Nutrient agarNo. 2	37 °C/ 24-48 hours
CCM 1944	*Proteus mirabilis*	Nutrient agar No. 2/DC agar	37 °C/ 24-48 hours
Clinical isolate	*Staphylococcus saprophyticus*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 5693	*Arcanobacter haemolyticus*	Mueller-Hinton + 5% ram's blood	37 °C/ 24-48 hours
CCM 4699	*Listeria monocytogenes*	blood agar with 7% ram blood	37 °C/ 24-48 hours

Table 2: Results of dilution (suspension) method

Microorganisms		**Control of initial concentration (cfu/ml)**	**OKCEL After 6 hours (cfu/ml)**	**OKCEL After 24 hours (cfu/ml)**	**OKCEL After 48 hours (cfu/ml)**
CCM 4223	*Staphylococcus aureus*	$1{,}0 \cdot 10^8$	0	0	0
CCM 4418	*Staphylococcus epidermidis*	$3{,}9 \cdot 10^8$	0	0	0
Clinical isolate	*Streptococcus pyogenes group A*	$1{,}2 \cdot 10^8$	0	0	-
CCM 6187	*Streptococcus agalactiae group B*	$1{,}5 \cdot 10^8$	0	0	-
CCM 4046	*Streptococcus salivarius*	$4{,}3 \cdot 10^7$	0	0	-
Clinical isolate	*Branhamella catarrhalis (Moraxella)*	$3{,}4 \cdot 10^7$	0	0	-
CCM 3954	*Escherichia coli*	$1{,}4 \cdot 10^8$	$2{,}2 \cdot 10^2$	0	0
Clinical isolate	*Klebsiella pneumoniae*	$1{,}0 \cdot 10^8$	$5{,}0 \cdot 10^3$	0	0
CCM 4420	*Salmonella enteritidis*	$1{,}3 \cdot 10^8$	$2{,}7 \cdot 10^2$	0	0
CCM 303	*Serratia marcescens*	$6{,}7 \cdot 10^8$	0	0	0
Clinical isolate	*Methicillin-resistant Staphylococcus aureus (MRSA)*	$6{,}9 \cdot 10^7$	$2{,}6 \cdot 10^6$	0	-

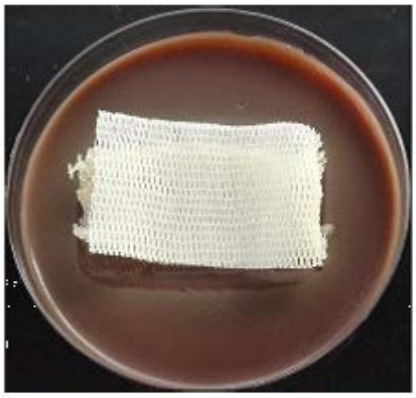
Clinical isolate	*Vankomycin-resistant Enterococcus (VRE)*	$5{,}4 \cdot 10^7$	$5{,}8 \cdot 10^6$	$1 \cdot 10^1$	0
Clinical isolate	*Methicilin-resistant Staphylococcus epidermidis (MRSE)*	$8{,}5 \cdot 10^7$	$6{,}3 \cdot 10^6$	0	0
CCM 1799	*Proteus spp.*	$2{,}0 \cdot 10^8$	$8{,}2 \cdot 10^2$	$3{,}2 \cdot 10^2$	0
CCM 1729	*Corynebacterium xerosis*	$5{,}9 \cdot 10^7$	0	0	-
Clinical isolate	*Mycobacterium smegmatis*	$3{,}0 \cdot 10^6$	N	$5{,}2 \cdot 10^2$	0
CCM 4435	*Clostridium perfringens*	$2{,}3 \cdot 10^7$	0	0	0
CCM 4508	*Bacteroides fragilis*	$5{,}5 \cdot 10^7$	0	0	-
CCM 4224	*Enterococcus faecalis*	$1{,}4 \cdot 10^8$	N	0	0
CCM 1903	*Enterobacter cloacae*	$1{,}3 \cdot 10^8$	0	0	0
CCM 3955	*Pseudomonas aeruginosa*	$1{,}0 \cdot 10^8$	0	0	0
CCM 2660	*Pseudomonas stutzeri*	$4{,}6 \cdot 10^8$	0	0	0
CCM 1944	*Proteus mirabilis*	$2{,}6 \cdot 10^8$	$1{,}9 \cdot 10^2$	0	0
Clinical isolate	*Staphylococcus saprophyticus*	$7{,}0 \cdot 10^7$	$5 \cdot 10^1$	$1 \cdot 10^1$	0
CCM 5693	*Arcanobacter haemolyticus*	$2{,}3 \cdot 10^7$	0	0	0
CCM 4699	*Listeria monocytogenes*	$5{,}2 \cdot 10^8$	0	0	0

Table 3: Results of diffusion method

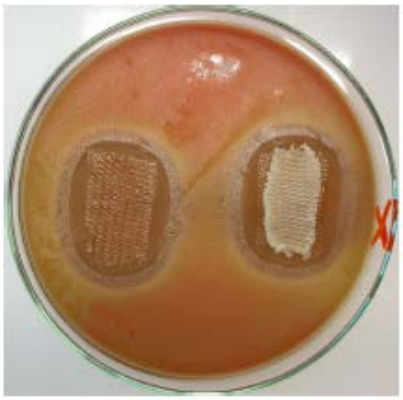
Microorganisms		**Zone of inhibition (mm)**
CCM 4223	*Staphylococcus aureus*	0
CCM 4418	*Staphylococcus epidermidis*	2
Clinical isolate	*Streptococcus pyogenes group A*	5
CCM 6187	*Streptococcus agalactiae group B*	5
CCM 4046	*Streptococcus salivarius*	4
Clinical isolate	*Branhamella catarrhalis (Moraxella)*	7
CCM 3954	*Escherichia coli*	2

Clinical isolate	*Klebsiella pneumoniae*	5
CCM 4420	*Salmonella enteritidis*	3
CCM 303	*Serratia marcescens*	4
Clinical isolate	*Methicillin-resistant Staphylococcus aureus (MRSA)*	4
Clinical isolate	*Penicillin-resistant Streptococcus pneumoniae (PRSP)*	5
Clinical isolate	*Vankomycin-resistant Enterococcus (VRE)*	5
Clinical isolate	*Methicilin-resistant Staphylococcus epidermidis (MRSE)*	6
CCM 1999	*Bacillis subtilis*	5
CCM 1799	*Proteus spp.*	5
CCM 1729	*Corynebacterium xerosis*	7
Clinical isolate	*Mycobacterium smegmatis*	0
CCM 4435	*Clostridium perfringens*	2
CCM 4508	*Bacteroides fragilis*	7
CCM 4224	*Enterococcus faecalis*	5
CCM 1903	*Enterobacter cloacae*	2
CCM 3955	*Pseudomonas aeruginosa*	1
CCM 2660	*Pseudomonas stutzeri*	5
CCM 1944	*Proteus mirabilis*	2
Clinical isolate	*Staphylococcus saprophyticus*	7
CCM 5693	*Arcanobacter haemolyticus*	9
CCM 4699	*Listeria monocytogenes*	0

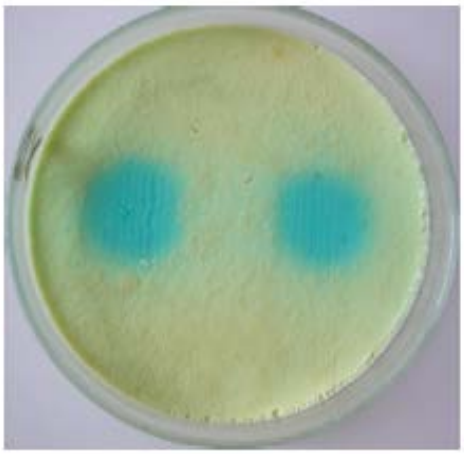
Chosen Photos - diffusion method results in selected bacteria



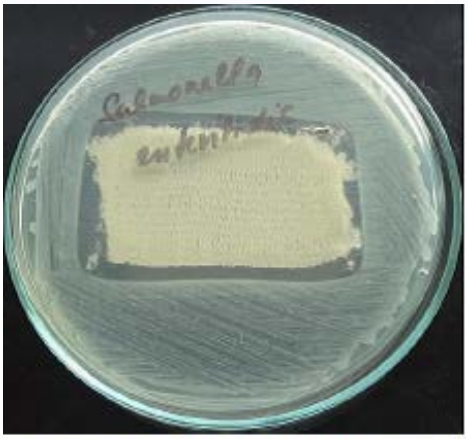
Clostridium perfringens
(Nutrient agar No. 2 with 5 % sheep defibrinated blood)



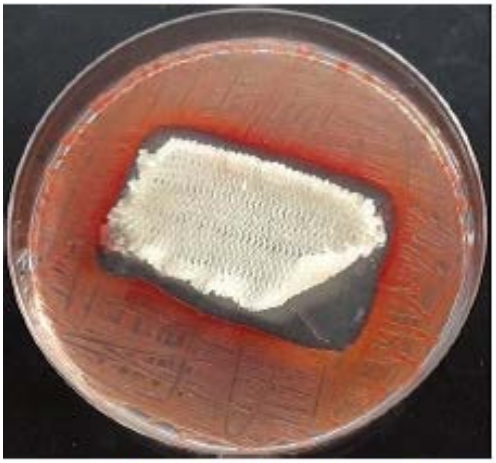
Methicilin-resistant Staphylococcus aureus (MRSA)
(Mueller Hinton Agar with 5 % sheep defibrinated blood)



Mycobacterium smegmatis
(Lowenstein-Jensen medium)



Salmonella enteritidis
(Nutrient agar No. 2)



Serratia marcescens
(Nutrient agar No. 2)

Proteus spp.
(Nutrient agar No. 2)

Conclusion:

Dilution (suspension) method:
OKCEL has an inhibitory (bactericidal) effect on almost all tested microorganisms. The density of the majority bacterial strains was reduced by 7-8 logarithmic degrees already after six hours of exposure. Density of clinical isolates of bacterial strains resistant to antibiotics was reduced after 24 hours of OKCEL's exposure. The least effective was OKCEL while interacting with *Mycobacterium smegmatis* – it reduces the number of cells by 4 logarithmic degrees after 24 hours.

Diffusion method:
The inhibition zones around the tested samples of OKCEL were observed in almost all tested microorganisms except for *Staphylococcus aureus*, *Mycobacterium smegmatis*, and *Listeria monocytogenes*. However, neither of these bacterial strains growth was observed under the tested material OKCEL, which is proving the inhibitory effect and also the bacteriostatic properties.

OKCEL tested by both dilution and diffusion methods showed an enhanced antimicrobial (bactericidal) activity as well as the bacteriostatic properties. No bacterial growth and/or presence in contact with OKCEL sample (on/under) were verified for wide range of bacterial strains. Results of this study have proven that OKCEL has great potential to be used as a dressing material in medical practice.

References:
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Dineen P.: The effect of oxidized regenerated cellulose on experimental intravascular infection. *Surgery*, 1997, 82(5): 576-9.
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