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Cytotoxicity testing of burn wound dressings: first results

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Abstract Topical antimicrobial therapy represents an essential part of burn wound care. In order to prevent and treat burn wound infection dressings with antimicrobial properties are applied directly on the wound surface. Not only the infection control but also promotion of healing is very important in burn wound management. It is well known, that a dressing in bactericidal concentration might also delay wound healing. This study was aimed to evaluate the potential toxic effect of topical antimicrobial agents on murine and human dermal cells. For toxicity testing the method by Vittekova et al. was used to evaluate potential toxic effects of 16 agents and 6 control samples on two in vitro cultured cell systems [3T3 cells and dermal fibroblasts] during the first 24 h. Following the 24 h cell culture with the tested agents the live cell counts were evaluated. According to results obtained on both cell systems, the tested samples were divided into three groups—nontoxic, semi-toxic and toxic. Nontoxic samples included Acetic acid 1%, Acticoat®, Dermacyn®, Framykoin®, Silverlon®, gauze, acellular human allodermis and acellular porcine xenodermis. Semi-toxic group included Algivon®Plus, Aquacel®Ag, Betadine®, Nitrofurazone, Octenisept®, Suprasorb® A and a porcine dermal scaffold Xeno-Impl. Finally, the toxic group included Algivon®, Dermazin®, Ialugen®Plus, Prontoderm®, Suprasorb® A Ag and 20% SDS. As the preliminary results of this study have shown, our findings may serve as a potential guide to selection of the most appropriate topical antimicrobial dressings for treatmet of burns. However before they can be translated into clinical practice recommendations, more research on antimicrobial dressings cytotoxicity testing will be necessary.

Keywords Topical antimicrobials · Cytotoxicity testing · Burn wounds

Introduction

Local infection and burn-wound sepsis are still some of the most severe problems in the treatment of thermally injured patients (Ryssel et al. 2009). It is now estimated that approximately 75% of the mortality following thermal injuries is related directly to infection (Guggenheim et al. 2011; Koller et al. 1999; Königová and Bláha 2010). Standard of care at specialized burn centres worldwide is early excision of necrotic tissues with subsequent wound coverage, or closure, which results in decreased mortality (Herndon et al. 1989). Other essential part of burn wound care is application of topical antimicrobial-wound agents. They can be used routinely almost at all stages of wound care. Another benefit of topical agents is that they are applied directly to the site of

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colonization or infection and can be used for both prophylaxis and treatment of burn wound infection (Davis et al. 2008). According to European practice guidelines for burn care published by European Burns Association, there is no clinical directive evidence to support the choice of one dressing over another (European Burns Association 2013). The current range of commonly used topical agents include topical antibiotics (e.g. bacitracin, mupirocin, neosporin, polymyxin B, nitrofurazone, nystatin), different silver impregnated dressings, honey based dressings, iodine solutions, chitosan preparations, acetic acid solution and others (Dai et al. 2010). In 1970s Fox (1975) combined silver nitrate with a sulphonamide derivate—sulfadiazine in a water soluble cream for topical use. The final product—silver sulfadiazine cream (SSD) benefits from the inhibitory effect of silver and the antibacterial action of sulfadiazine. SSD became in the last decades one of the most commonly used topical antibacterial agents for deep burns (Hermans 1998, 2007). SSD has been used often as a reference standard therapy in many studies (Fuller 2009; Daryabeigi et al. 2010). Its antimicrobial activity especially against aerobic Gram-negative bacteria is well known, however recent studies indicate, that SSD may delay wound healing (Rosanova et al. 2012). Many studies are focused on finding a better alternative to SSD. As showed recently, honey was found to have statistically significant beneficial effects compared to SSD for the outcomes time complete wound healing, proportion of wounds completely healed and proportion of infected wounds rendered sterile (Aziz and Hassa 2017). Despite of rich variety of silver impregnated dressings also antimicrobial solutions may be used in burn wound care (Fraise et al. 2013; Kapur and Marwaha 2011). Study of Kapur et al. showed better healing of burns treated by superoxidised solution compared with povidone iodine solution. Other specialists recommend acetic acid for its good antimicrobial activity and low costs (Fraise et al. 2013). In general, antimicrobial properties of different topical agents for burn wound carehave been well reported (Hammond et al. 2011; Boekema et al. 2013; Uygur et al. 2009; Hajská et al. 2014) but their potential side effects and cytotoxicity should not be forgotten (Dai et al. 2010; Atiyeh et al. 2007; Vandamme et al. 2013; Vermeulen et al. 2010; Nasgoba et al. 2013). In appropriate burn wound care it is essential to know, whether a topical agent is really bactericidal and therefore suitable especially for deep burns containing a lot of necrotic tissue or whether it is both bactericidal and noncytotoxic thus causing no prolongation of burn wound healing time.

Aim

The aim of this study was to evaluate the potential toxic effect of topical antimicrobial agents on cultured murine and human dermal cells. The study was designed to test various agents in different application forms—solutions, creams, ointments and commercially produced impregnated dressings as well. Selected agents are routinely used in our burn centre or recommended for burn wound care.

Materials and methods

Isolation and cultivation of cells

3T3 NIH murine fibroblasts (3T3) were purchased from the ECCACC collection (Lambda life, ECCACC umber 850 22,108) and cultivated in D-MEM (Dulbecco—modified medium, PAN BIOTECH—GERMANY) supplemented with 10% of FCS (foetal calf serum, PAN BIOTECH—GERMANY).

Dermal fibroblasts (DF) were obtained from human skin dermal explant cultures. After isolation of keratinocytes, the remainder (dermis) of each skin graft procured for keratinocyte cultureswas dried in a sterile 6 cm Petri dish for cell cultures for 20 min, after which a cell cultivation medium (D-MEM + 10% FCS—foetal calf serum) was added. DF started to grow from explants after 3–5 days, whereupon they were isolated with trypsin and seeded into cultivation flasks. The cultivation medium was changed every second day (Dragúňová et al. 2013).

Both 3T3 and DF cells were routinely cultured according to standard procedures (incubated in humidified air at 37 °C with 7.0% CO_2) and passaged at least once a week.

Tested agents

1. Wound dressings samples—16 topical antimicrobial agents in the same application forms as used for patients treatment were tested (dressings tested

Table 1 Topical agents tested

Solutions	Agent	
	Acetic acid 1% solution	Produced by hospital pharmacy
	Betadine® EGIS solution Pharmaceuticals PLC	Povidon iodine 100 mg/ml solution
	Dermacyn® Oculus solution	Super-oxidized solution
	Nitrofurazone 0.2% solution	Produced by hospital pharmacy
	Octenisept® Schülke and Mayr GmbH solution	Octenidindihydrochlorid 0.1/100 g, Phenoxyethanol 2.0/100 g
	Prontoderm® B.Braun solution	Polyhexametylenbiguanid <1%
Creams/ ointments	Dermazin® SANDOZ crm.	1% silver sulfadiazine
	Framykoin® ZENTIVA ung.	Neomycin, bacitracin
Commercial dressings	Acticoat®Smithandnephew	Nanocrystalline silver impregnated pad; it consists of 3 layers: an absorbent rayon/ polyester inner core sandwiched between outer layers of nanocrystalline silvercoated, low adherent polyethylene net
	Algivon® ADVANCIS MEDICAL	Alginate dresing impregnated with 100% manuka honey
	AlgivonPlus® ADVANCIS MEDICAL	Reinforced alginate dresing impregnated with 100% manuka honey
	Aquacel®Ag CONVATEC	Ionic silver impregnated pad; non- woven pad or ribbon dressing composed of sodium carboxymethylcellulose and 1.2% ionic silver
	Ialugen®Plus IBSA	1% silver sulfadiazine, +0.2% natrii hyaluronas
	Silverlon®(BPD-44) ARGENTUM MEDICAL	Silver plated nylon technology (546 mg metallic silver/100 cm^2)
	Suprasorb®A Lohmann and Rauscher	Calcium alginate wound dressing
	Suprasorb® A Ag Lohmann and Rauscher	Calcium alginate wound dresing with silver

are listed in Table 1). Solutions, creams and ointments were applied on sterile gauze. Commercially produced impregnated dressings were used according to the producers' recommendation. All of the samples were cut into 1 cm^2 pieces under sterile conditions. In order to saturate sterile gauze pieces 100 µl of each solution and/or 50 µg of creams/ointments were applied.

2. Control samples:
   (a) No agent.
   (b) Sterile gauze.
   (c) 20% Sodium dodecile sulphate (SDS).
   (d) Human acellular allodermis prepared in The Central Tissue Bankof University Hospital Bratislava (CTB) (prepared according to the CTB standard operative procedures).
   (e) Porcine acellular xenodermis prepared in the CTB prepared according to the standard procedure protocols).
   (f) Xeno-Impl (Institute of Biomedical Technologies BiomedičnychTernopil State Medical University, Ukraine)—commercially available porcine acellular dermal matrix.

Cytotoxicity testing

For cytotoxicity testing the method by Vittekova et al. (2014) was used. 3T3 and DF cells were cultured as described above until achieving confluence. Cells morphology was confirmed microscopically before application of the tested agents. 1cm^2 of tested wound dressings and control samples were applied into Petri dish on confluent cultured cells and their cultivationat 37 °C

with 7% CO_2 continued thereafter for 24 h. After removal of the tested agents/controls samples the dishes were washed with phosphate buffered saline (PBS) and ethylenediaminetetraacetic acid (EDTA) and then the cells were trypsinized. Cell counts were provided in a Bürker's counting chamber. The numbers of cells adherent to the surface of Petri dish following the 24 h cultivation of cells covered with tested samples were compared with numbers of adherent cells following 24 h culture with no agent. The percentage of adherent cells was calculated. All the test samples and controls were tested in triplicates. The experiments have been carried out first with 3T3 cells and then repeated with DF.

Cytotoxic effect was evaluated by counting the numbers of cells that remained adherent to the culture surface following the 24 h. culture with each sample. The cell counts were compared with the number of adherent cells following the 24 h. culture with no agent. Calculation of percentage of adherent cells compared with those in the controls groups was performed.

Results

100% was represented by the count of adherent cells in the control group with no agent (Tables 2, 3, 4, and 5).

Table 2 3T3-cell counts following the action of the tested agents

CELLS: 3T3 Agents	3T3 cells in number	3T3 cells in %
AA1%	9.60E+05	96
Acticoat	7.70E+05	77
Algivon	8.00E+04	8
AlgivonPlus	7.20E+05	72
AquacelAg	6.00E+05	60
Betadine	6.00E+04	6
Dermacyn	1.00E+06	100
Dermazin	1.00E+04	1
Framykoin	9.20E+05	92
Nitrofurazone	5.40E+05	54
IalugenPlus	1.00E+05	10
Octenisept	7.70E+05	77
Prontoderm	1.20E+05	12
Silverlon	8.10E+05	81
Suprasorb A	1.00E+06	100
Suprasorb A Ag	1.90E+05	19

Table 3 3T3-cell counts following the action of the control samples

CELLS: 3T3 Control samples	3T3 cells in numbers	3T3 cells in %
A. aloderm	1.00E+06	100
A. xenoderm	1.00E+06	100
Xeno-Impl	3.30E+05	33
20% SDS	0.00E+00	0
Gauze	9.00E+05	90
No agent	1.00E+06	100

Table 4 DF-cell counts following the action of the tested agents

CELLS: dermal fibroblasts Agents	DF cells in number	DF cells in %
AA1%	7.70E+05	100
Acticoat	6.70E+05	87
Algivon	3.00E+04	3.9
AlgivonPlus	5.70E+05	74
AquacelAg	3.80E+05	49
Betadine	4.20E+05	55
Dermacyn	6.40E+05	83
Dermazin	2.00E+04	2.6
Framykoin	6.50E+05	84
IalugenPlus	1.60E+05	20
Nitrofurazone	3.20E+05	42
Octenisept	1.65E+05	21
Prontoderm	7.00E+04	9
Silverlon	7.70E+05	100
Suprasorb A	3.40E+05	44
Suprasorb A Ag	8.00E+04	1

Table 5 DF-cell counts following the action of the control samples

CELLS: dermal fibroblasts Control samples	DF cells in number	DF cells in %
A. aloderm	7.30E+05	95
A. xenoderm	7.70E+05	100
Xeno-Impl	3.85E+05	50
20% SDS	0.00E+00	0
Gauze	7.70E+05	100
No agent	7.70E+05	100

1. Wound dressings samples

- Nontoxic agents—higher adherence then 70% on both cell systems was observed in the dressings Acetic acid 1%, Acticoat®, Algivon®Plus, Dermacyn®, Framykoin® and Silverlon®.
- Semi toxic agents—adherence between 70 and 20% on both eventually on one of tested cell systems was observed in 7 agents—Aquacel®Ag, Betadine®, Nitrofurazone, Ocetnisept®, Suprasorb® A.
- Toxic agents—smaller adherence then 20% on both cell systems was observed in the dressings Algivon®, Dermazin®, Ialugen®Plus, Prontoderm® and Suprasorb® A Ag.
- Control samples—sterile gauze, acellular alodermis and acellular xenodermis were according to evaluation on both systems nontoxic, 20% SDS was toxic and scaffoldXeno-Impl can evaluated as semitoxic.

As for the majority of the 22 tested samples, a concordance in both cell systems was observed. However a difference was observed in 3 tested agents (Betadine® 6% in 3T3/55% in DF, Octenisept® 77% in 3T3/21 in DF and Suprasorb® A 100% in 3T3/44% in DF). In the case of Octenisept® and Suprasorb® A this can be explained with higher sensitivity of DF. On the other hand, in the case of Betadine® solution much more DF cells remained adherent to the dish surface then 3T3. Therefore the microscopic evaluation was performed very preciously. Significant morphological changes in tested DF with Betadine® compared to DF control samples were observed. Comparing the cell morphology by microscopy—oval shape of dermal fibroblasts and their morphology did not correspond to normal morphology. Morphological changes of 3T3 cells following the application of toxic agent are visible in Fig. 2, meanwhile Fig. 1 shows normal 3T3 cells and morphological changes of DF cells following application of semitoxic Xeno-Imp. The changes are visible in Fig. 4, meanwhile Fig. 3 shows normal DF.

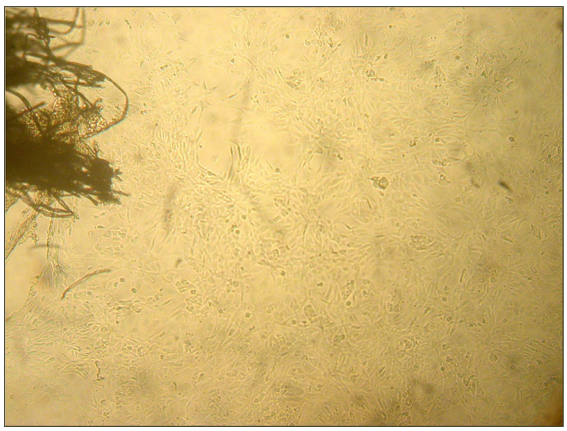

Fig. 1 Normal 3T3 cells—following the 24 h action of sterile gauze

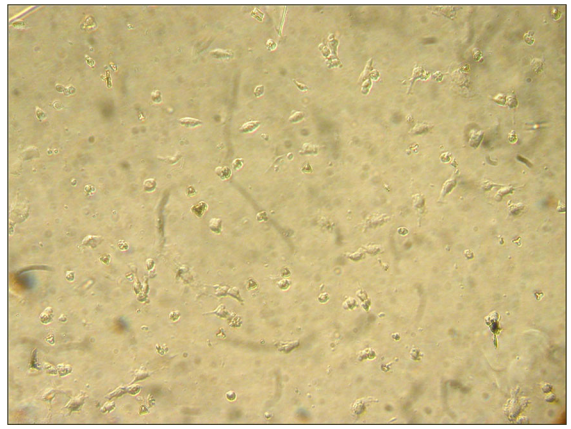

Fig. 2 Morphologically changed 3T3 cells—following the 24 h action of toxic agent (Betadine®)

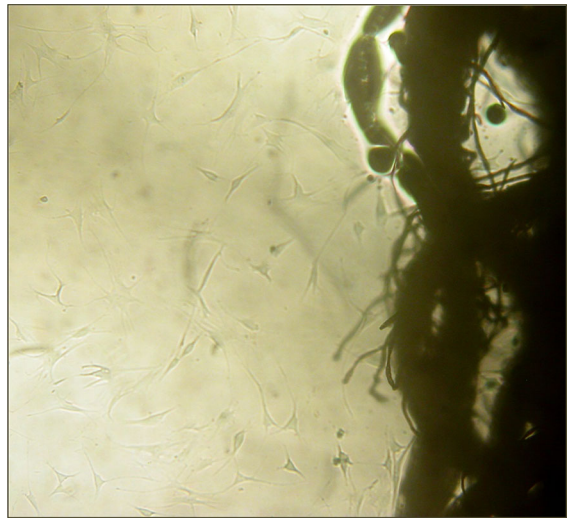

Fig. 3 Normal DF cells—following the 24 h action of sterile gauze

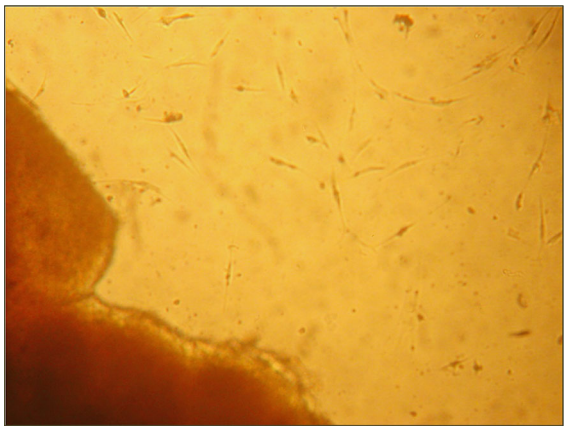

Fig. 4 Morphologically changed DF cells—following the 24 h action of semitoxic Xeno-Impl

Figures 5 and 6 show all the results (for tested agents and control samples) in both the cell systems.

Discussion

Both 3T3 and DF cells were used to evaluate the cytotoxicity of wound dressings and control samples. Use of two cell types of differing sensitivities was intended to ensure detection of cytotoxic effects that might not have been picked up in only a single cell system was employed. This study was aimed to observe a potential cytotoxicity of 16 topical antimicrobial agents and 6 control samples. All the tested topical antimicrobial agents were used in the same application forms (e.g. solutions, creams, impregnated dressings) and concentrations as are used for burn wound treatment in our department. Two cell systems—3T3 and DF cells were used to evaluate the cytotoxicity of the tested topical agents and control samples. Use of two cell types of differing sensitivities was intended to ensure detection of cytotoxic effects that might not have been picked up in only a single cell system was employed. The results were evaluated as numbers of cells following the 24 h cell culture with the tested agent. According to results obtained of both cell systems, the tested samples were divided into three groups—nontoxic, semi-toxic and toxic. To the nontoxic samples belonged the dressings Acetic acid 1%, Acticoat®, Algivon®Plus, Dermacyn®, Framykoin® and Silverlon® and also sterile gauze, acellular alodermis and acellular xenodermis. To the semitoxic group belonged Aquacel®Ag, Betadine®, Nitrofurazone, Ocetnisept®, Suprasorb® A and the scaffold Xeno-Impl. Finally to the toxic group belonged the dressings Algivon®, Dermazin®, Ialugen®Plus, Prontoderm® and Suprasorb® Ag and 20%

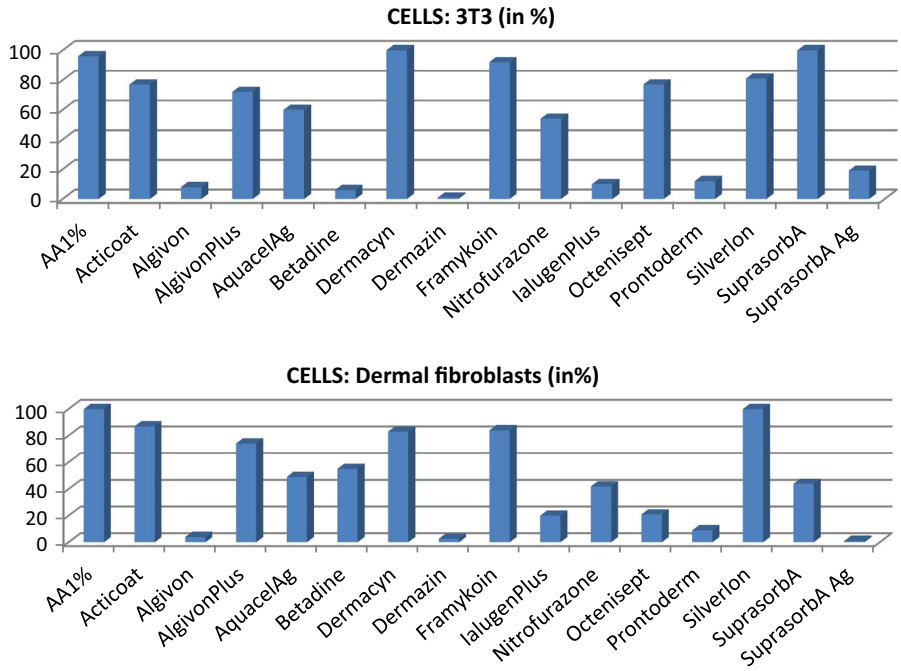

Fig. 5 Cell counts following the 24 h action of the tested agents

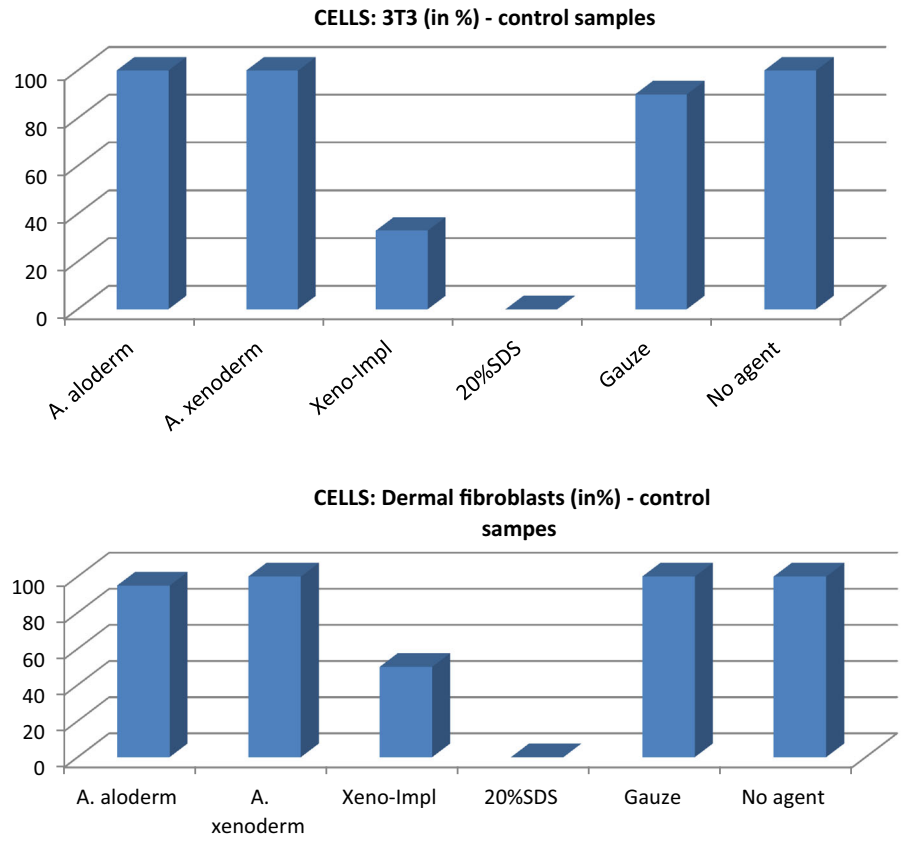

Fig. 6 Cell counts following the 24 h action of the control samples

SDS. Six of the tested 16 antimicrobials are silver-based and in burn wound treatment belong to the standard of care. Laboratory studies confirmed that both keratinocytes and fibroblasts are susceptible to lethal damage when exposed to concentrations of silver which are lethal for bacteria (Poon and Burd 2004). Other studies confirmed that irrespective of the form of silver delivery, its cytotoxicity is directly proportional to its concentration (Abe et al. 2003). Our study showed interesting results with tested silver-based dressings. Two agents (Acticoat® and Silverlon®) were nontoxic and one agent (Aquacel®Ag) was semi-toxic. Finally three (Dermazin®, Ialugen®Plus and Suprasorb® Ag) were toxic; meanwhile the most toxic agent was Dermazin®—1% silver sulfadiazine cream.

Another promising type of burn wound therapy is represented by honey-based dressings because of their antibacterial effect and healing stimulating properties (Vandamme et al. 2013; Subrahmanyanm 1993, 1998; Malik et al. 2010). In our study two honey-based agents were included, and they were evaluated as non-toxic (Algivon®Plus) and toxic (Algivon®). The possible reason might be a different content of honey in each dressing.

As for the majority of the 16 topical antimicrobial agents and 6 control samples a concordance in both cell systems was observed. However 3 agents (Betadine®, Octenisept® and Suprasorb® A) showed a big difference in the percentage of adherenceobserved. In the cases of two agents (Octenisept® 77% in 3T3/21 in DF and Suprasorb® 100% in 3T3/44% in DF) a higher sensitivity of DF to the tested agents was observed. Interestingly in the case of Betadine® solution (6% in 3T3/55% in DF) much higher sensitivity of 3T3 cells was observed. The microscopic evaluation of both cell systems influenced by Betadine® solution was performed very preciously. The changes of cell morphology were observed—normal cell shape was changed into an atypical oval shape (Figs. 1, 2, 3, 4). As for iodine dressings they are believed to influence tissue regeneration negatively due to a toxic effect on the

host cells, but Vermeulen et al. (2010) concluded that there is available evidence supporting no delay of wound healing process. It would be therefore optimal to continue with cytotoxicity testing of different iodine dressings using also other methods.

As for control samples, they were selected in order to confirm the suitability of the testing method. Culture with no agent was used to count % of adherence so that it represented 100% percent. Sterile gauze was tested in order to confirm its nontoxicity, because many agents (solutions, creams, ointments) are applied on it. 20% SDS served as toxic control. As for human acellular allodermis and porcine acellular xenodermis prepared in our Tissue Bank, they were used to confirm their nontoxicity and Xeno-Impl was tested in order to confirm its toxicity, which was shown by a previous study realized in our Tissue Bank (Vitteková et al. 2014).

It can be presumed, that the lower percentage of cell adherence can be in concordance with cytotoxic effect of the agent. In order to confirm this hypothesis we plan to use other in vitro methods to clearly demonstrate the cell vitality. The final results are aimed to give a recommendation for surgeons, which agents are due to their noncytotoxicity suitable for us in superficial burns and their conservative therapy.

Conclusions

Topical antimicrobial therapy represents an essential part of burn wound care. Wound dressings are applied directly on the wound surface and thanks to their antimicrobial properties can prevent and treat wound infection. Another important feature of the dressing should be the promotion of healing. However it is known, that a dressing in bactericidal concentration might also delay wound healing because of its cytotoxicity. This study was aimed to evaluate the potential toxic effect of 16 wound dressings and 6 control samples (including no agent) on murine and human dermal cells. Results showed toxic (6 samples), semi-toxic (6 samples) and non-toxic (9 samples) effect of the tested samples on both cell systems. Especially the nontoxic ones (Acetic acid 1%, Acticoat®, Algivon®Plus, Dermacyn®, Framykoin® and Silverlon® and also sterile gauze, acellular alodermis, acellular xenodermis) can be recommended for application on superficial burns. However in order to contribute positively to the choice of proper dressing in concrete clinic situation we would like to continue with this research using also other testing procedure.

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