IMPACT OF FABRICS FROM TRANSGENIC FLAX PLANT ON HUMAN DERMAL FIBROBLASTS IN VITRO PROLIFERATION

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Abstract: Previously it was documented that transgenic flax plants, which contained an increased level of polyphenolic compounds, significantly improved healing of skin wounds lesions. In order to recognize mechanisms of beneficial action of transgenic flax fabrics on wound healing, in the present study the impact of flax fabric pieces/cuts from three types of transgenic flax on normal human dermal fibroblasts primary culture (NHDF) was investigated. NHDF cell cultures were exposed for 48 h to specific area of flax fabric cuts, made from M50, B14 and M50+B14 (intertwined fibers of M and B), or parallely, extracts from fibers of the tested flax materials to cell culture medium. Cultures were inspected for cell viability, proliferation, cell cycle changes and for their resistance to oxidative stress (consecutive addition of H2O2 to harvested cell cultures). None of the tested flax fabrics were cytotoxic to fibroblast cultures and also did not increase significantly a frequency of apoptotic cells in cultures. In the comet assay, the tested flax fabrics revealed significant protective effect on DNA damage caused by addition of H2O2 to the cultures at the end of incubation time. Fabrics from transgenic flax significantly enhanced fibroblasts proliferation in vitro estimated with the SRB test. Flow cytometric analysis revealed higher frequency of cells in the S phase, in the presence of transgenic flax fabrics. Fabrics from B14 and M50+B14 flax are the most potent activators of NHDF cells in applied in vitro tests, hence they could be recommended for elaboration of new type bandage, able to improve skin wound healing.

Keywords: fabrics from transgenic flax, fibroblasts, proliferation, oxidative stress resistance

Flax (*Linum usitatissimum* L.) is a crop plant traditionally cultivated as a source of fibers and oil. The plant has been genetically modified in order to enhance wound healing properties of the fibers (1, 2) providing stable overexpression of enzymes for polyhydroxybutyrate (PHB) synthesis (1), and enzymes of phenylpropanoid pathway (3). New obtained flax types, called M type and B type composite fibers, were previously found to promote proliferation rate of cultured fibroblasts (4). The transgenic flax fibers accumulate phenolic acids and flavonoids with antioxidant activity (2). It was reported in pre-clinical trials that wound dressings made of fibers from transgenic flax significantly improved healing of chronic skin ulcers (5, 6). The impact on a healing wounds needs further studies on action of compounds released from flax fibers to wound environment and its nearest surrounding on biological activity of cells engaged in skin regeneration and repair. It was well documented in the literature that an activation and cross-stimulation of keratinocytes and dermal fibroblasts are necessary for skin lesions reparation (7–9). Fibroblasts activation is crucial for wound healing, since various compounds secreted by activated fibroblasts, as numerous cytokines, chemokines and growth factors, play a key role in wound repair and scar formation (10–13). In this paper, we describe an impact of fabrics produced from fibers of transgenic flax (B, M) and from non-transgenic cultivar ‘Nike’ on human primary skin fibroblast cultures. Results obtained with biological tests were compared by means of the multi-criterial analysis procedure (14, 15). According to the procedure, total beneficial effect on human dermal fibroblasts was calculated for each tested flax fabric and the most favorable flax fabrics, able to improve wound healing, were picked up as candidates for new type bandage.

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EXPERIMENTAL

Chemicals
Sulforhodamine (SRB), low melting point agarose (Sigma type VII) at 37°C, regular agarose (Sigma type I-A), the fluorescent dye – DAPI (4’,6-diamidino-2-phenylindole dihydrochloride), Trizma-base and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypsin EDTA solution was from Lonza (Verviers, Belgium). Cell culture plastic plates (24-wells) were purchased from Lonza (Verviers, Belgium). Alexa Fluor 488 Annexin V/Dead Cell Apoptosis Kit and the Live/Dead Cell Imaging Kit together with propidium iodide (PI) powder were purchased from Thermo Fisher Scientific (Waltham, MA, USA).FITC BrdU Flow Kit was obtained from BD Pharmingen, (San Jose, CA, USA). Phosphate buffered saline (PBS), 0.4% trypan blue solution and all other chemicals were obtained from POCh (Gliwice, Poland).

Cell culture media
DMEM medium and fetal bovine serum (FBS) were purchased from PAN-Biotech GmbH (Aidenbach, Germany). The solution of antibiotics (10,000 U/mL penicillin, 10,000 µg/mL streptomycin) containing 29.2 mg/mL L-glutamine (100×) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cells
Normal human dermal fibroblasts (NHDF) from adult donor, purchased from Lonza (Verviers, Belgium), and mice fibroblasts BALB/3T3 (clone A31 from mouse embryo donor, HPA Culture Collection) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The cells were grown at a density of 2 × 10⁶ cells/well in DMEM (containing 4.5 g/L of glucose) with 10% FBS, 2 mM L-glutamine and the antibiotics penicillin (100 U/mL) and streptomycin (0.1 mg/mL).

Cell culture conditions
Cells were grown in the recommended culture media in CO₂-incubator at 37°C and subcultured every 3 days. Before the test, adherent cells were detached with the trypsin EDTA solution, washed with PBS, spun down, counted, stained with a 0.4% solution of trypan blue, and inspected under a microscope for cell viability. Then, cells were plated on 24-well plastic culture plates (2 × 10⁶ cells/well) and incubated at 37°C in a CO₂-incubator for 24 h. afterwards, the tested flax fabrics or flax fibers extract were added, and the cultures were incubated for 48 h in CO₂-incubator at 37°C. Then, cells were harvested and intended for further tests, as given below.

Plants
The transgenic flax plants were generated by transforming Linum usitatissimum L., fibrous cultivar Nike as described previously (1, 16–18). The B14 transgenic plants were obtained by transforming Nike cultivar using the plasmid pGAglubsens, containing cDNA encoding β-1,3-glucanase from potato (GenBank: AJ586575.1) (18). B14 plants have ectopic expression of potato β-1,3-glucanase gene and exhibited significantly lowered level of monosaccharides, fatty acids and organic acids, and increased level of different amino acids, polyamines and antioxidants (18, 19). To obtain the M type flax cultivar Nike was transformed using construct containing β-ketothiolase (phb A), acetoacetyl-CoA reductase (phb B), and poly-3-hydroxybutyric acid synthase (phb C) from Ralstonia eutropha (17). The introduction of polyhydroxybutyrate synthesis genes into flax genome, yields fibers that contain higher quantities of phenolics in addition to polyhydroxybutyrate (20). B14 and M50 transgenic plants as well as fibrous Nike cultivar were grown in fields near Wroclaw in 2015 and were harvested after 4 months. The collected plants were retted by “dew method” and the fibres were obtained using standard technology and made into yarn without chemical processing. For production of fabrics, standard weaving method was used to obtain fabrics from plants type B and type M. In the case of M+B fabric an equal proportion M and B plants fibers were intertwined to form the fabric. The density of final flax fabrics was 220 g/m².

Preparation of flax fabrics and extract from flax fibers for biological tests
Fabrics were autoclaved, cut into pieces of 0.5–2.0 cm² surface area, soaked in culture medium (without FBS) and then located in 24-well plastic culture plates containing the tested cells monolayer. Fabric cuts floated just below the surface of culture medium.

Unlike fabrics, flax fibers, drowned in cell culture medium and closely covered cell monolayer, decreasing cells viability. Therefore, an indirect, two-step procedure was applied to study the impact of flax fibers on cells: at first, flax fibers, weighing 40 mg (equivalent of fabric cuts mass of 2 cm² surface), were soaked for 48 h in cell culture medium (without FBS), extracts containing compounds released from fibers during soaking procedure, were
added to cell cultures in a final volume of 0.25–1 mL (corresponding to 0.5-2.0 cm² of flax fabric cuts area). In this paper, the term “flax fibers extract” will be used to describe a conditioned medium of fibers soaked in DMEM (without FBS).

**Methods**

**Estimation of selected compounds in flax fabrics**

For phenolics and lutein analysis fabric sample (1 g) was ground in a Retsch mill and extracted trice with methanol. The bound phenolics were then released from fabric residue by hydrolysis with 2 M NaOH at 37°C for 24 h and then extracted with ethyl acetate after acidification. The solvent was evaporated and samples resuspended in methanol and analyzed as described in (20).

For sterols estimation, the fabrics were extracted trice with chloroform and the derivatization and analysis by GS-FID was done as described previously (21).

The isolation and fractionation of polyamines was performed as described previously (22). Briefly, 100 mg of ground fabrics were extracted with perchloric acid, hydrolyzed with 6 M HCl to release free polyamines, and subsequently dansylated to facilitate identification. The samples were analyzed using a Waters Acquity UPLC system with a 2996 PDA detector on an Acquity UPLC BEH C18, 2.1 × 50 mm, 1.7 µm column. The results were standardized with mixtures of dansylated polyamine standards (putrescine, spermidine, spermine).

**Microscopic evaluation of cell viability**

Viability of NHDF and on BALB 3T3 cells was checked by microscopic examination with the trypan blue exclusion method. Cells detached with trypsin-EDTA solution were stained for 10 min with 0.4% aqueous solution of trypan blue and unstained (live) and stained (dead) cells were counted under a microscope.

**Flow cytometric estimation of live/dead cells**

Propidium iodide (PI) was added to samples of detached cells suspended in PBS, to the final concentration of 1 µg/mL of the dye. After 15-min incubation in a dark, the samples were examined with the CyFlow Cube8 flow cytometer (Portec-Sysmex, Görlitz, Germany) and fluorescence emission was measured using a 488 nm excitation laser lamp and 536/40 (BP) and 630 nm (LP) emission filters. The samples were analyzed with CyView software. Granulation, size and fluorescence intensity were recorded for 40,000-100,000 cells. Percentages of viable, apoptotic and necrotic (dead) cells were calculated from dot-plot graphs.

**Comet assay**

After 48 h culture with the tested flax materials, cells were incubated with the PBS containing H$_2$O$_2$ [200 µM] for 30 min at 4°C and cell suspensions were carried on the standard procedure of the comet assay (23). Finally, slides stained with the fluorescent dye (DAPI, 1 µg/mL) were evaluated under a fluorescence microscope (Nikon Eclipse E-600) with UV 1A filter block, and with the digital camera and computer the CometPlus 2.5 software (Theta System Electronics Gmbh, Gröbenzell, Germany). Seventy-five comets randomly found on each slide under a microscope were scored in the computer’s data file. The DNA content in the comet’s head (%) and the tail length of the comets were analyzed.

**Determination of cell density/cell proliferation**

Determination was done with the sulforhodamine B (SRB)-colorimetric assay (24). NHDF cells grown on plastic culture wells were fixed with cold TCA (final concentration 10%, w/v) for 1 h at 4°C, then washed four times with tap water and air-dried at room temperature. A mildly acidic SRB solution (0.4% dye solution in 1% acetic acid) was added to each well for 30 min at 25°C, and unbound stain was removed by rinsing with aqueous solution of 1% (v/v) acetic acid. Culture plates were then allowed to dry at room temperature. The SRB bound to the cellular proteins was dissolved in 10 mM Trizma-base solution (pH 10.5) for 10 min on a gyratory shaker and the absorbance of the SRB solution was estimated at 540 nm in a Victor 2 microplate reader (Perkin-Elmer, MA, USA).

**Evaluation of cell cycle**

Cell cycle of NHDF fibroblasts was determined with FITC BrdU Flow Kit, according to the procedure recommended by supplier. Cells were labeled with BrdU and incubated with fluorescent antibodies conjugated to FITC. Total amount of the
DNA in cells was determined by staining with the dye 7-amino-actinomycin D (7-AAD).

**Statistical analysis**

The results estimated in cultures with the presence of the tested flax materials were compared to the relative control cultures (cells cultured without flax materials) with the paired $t$-test.

The multi-criterial analysis (MCA) procedure was carried out according to literature data (14, 15) with own modifications. Briefly, the obtained results were compared to the relative control cultures $[E/E_0]$ and the statistical distances between the expected (exp.) and observed (obs.) results were calculated with the standard formula: $b = (\text{exp.} - \text{obs.})^2 / \text{exp.}$ The expected values in each test were assumed as

![Figure 1. Diagram showing location of flax fabric in the test-culture well](image)

![Figure 2. Frequency of apoptosis (early + late) in NHDF cell cultures after 48 h of incubation with flax fabrics or flax fibers extract.](image)

Statistical significance of the results in comparison to the control (K; cultures with no flax fabric / fibers) were calculated with the paired $t$-test ($^* p < 0.05$; $^{**} p < 0.01$)
the most favorable results observed for three flax fabrics. Results of the calculation were expressed as 1/b ratios and then multiplied by the indices of importance, arbitrary assumed to be potentially beneficial in skin wound healing. Finally, the results of the MCA procedure of compared fabrics were obtained according to equation:

\[ M = \sum \frac{1}{b} \]

Correlation between total favorable effects in the MCA procedure and contents of selected group of compounds in the tested flax fabrics was calculated with the Spearman’s rank correlation statistics.

RESULTS

Tested flax fabrics cuts placed in plastic cell culture wells floated just below the surface of medium and coated with an approximately 1 mm layer of the medium, as shown in Figure 1.

The scheme was drawn for 1 cm² pieces of the fabrics. For 2.0 cm² pieces, two cuts of 1 cm² area were placed in a cell culture well. Floating fabrics release several active compounds from flax fibers, also of polyphenolic compounds, as was confirmed by the HPLC analysis of polyphenolic contents in aqueous media after soaking flax fabrics (23).

Tested flax materials: both fabrics and flax fiber extracts did not influence on viability of NHDF and BALB 3T3 fibroblasts, as was confirmed by microscopic examination of cells stained with 0.4% trypan blue solution, and indirectly, by cytometric examination of propidium iodide (PI) staining. The results proved that the tested flax materials are not cytotoxic to fibroblasts in 48-hour cultures (data not shown).

Figure 3. Mean content of the DNA in the comets’ heads in NHDF cells incubated for 48 h with flax fabrics (A) or flax fibers extracts (B), then exposed to exogenous oxidant – H₂O₂ [200 µM, 20 min, 3°C]. The DNA content in the comets’ heads [%] was estimated in 75 nucleoids randomly found in the microscopic image. The results were presented as the mean ± SD; n = 6. K – control cultures exposed to H₂O₂ without previous incubation with flax materials. Statistical significance of the results, calculated with the paired t test, showed that all the results presented in Figure 4 were significant (p < 0.05 for each column).
Frequency of apoptosis in NHDF cultures after 48 h incubation with the tested flax materials is given in histograms in Figure 2.

Histograms in Figure 2A show that after incubation with the tested flax fabrics a frequency of apoptosis was slightly elevated in the case of B14 (up to 23%) and also of M50+B14 fabric (up to 25%), when compared to apoptosis frequency in the control culture (about 10%). However, it should be noted that frequency of apoptosis in NHDF cells cultured with genetically modified plants (M50, B14 and M50+B14 fabrics) did not reach statistical significance level, probably due to relatively high standard deviation of the measurements. The only significant value was calculated for Nike fabric (native, non-transgenic flax); in cultures with the highest area (2 cm²) of this fabric a frequency of apoptotic cells increased to 20%. Figure 2B shows that extracts from flax fibers significantly decreased frequency of apoptotic cells in NHDF cultures, when compared to frequency of “spontaneous” apoptosis found in the control cultures (10%). Also non-transgenic flax fibers extract (Nike) caused decrease of apoptotic cell numbers in NHDF cultures, however those results did not reach statistical significance level. Incubation of NHDF cells with the tested flax materials markedly increased resistance to exogenous oxidative stress. NHDF cultures incubated for 48 h with flax materials and subsequently exposed to high concentration of H₂O₂ [200 µM, 20 min, 3∞C] were subjected to oxidative DNA damage analysis using Comet assay. The comet assay reflected DNA strand breaks in the form of comet tails. Figure 4 shows that mean tail length of the comets in NHDF cultures carried out in the presence of flax fabrics (A) or flax fibers extracts (B) and consecutively exposed to exogenous oxidant – H₂O₂ [200 µM, 20 min, 3∞C]. The comet tails length was estimated in 75 nucleoids randomly found in the microscopic image. The results are given as the mean ± SD; n = 6. K – control cultures exposed to H₂O₂ without previous incubation with flax materials. Statistical significance of the results, calculated with the paired t test, revealed that all the results presented in Figure 4 were significant (p < 0.05 for each column).
3°C] exhibited markedly higher DNA content in the comets heads and lower length of the comets tails when compared to the relative control cultures. Results obtained with the standard comet assay are given in histograms in Figure 3.

Figure 3A shows that incubation of NHDF cells with the tested flax fabrics cuts caused an increase of the DNA content in the comets’ heads even by 29, 33 and 23% in cultures with the presence of M50, B14 and M50+B14, respectively. Also non-transgenic Nike flax fabric caused an increase of the DNA content in the comets’ heads by about 20%. Figure 3B shows that incubation of NHDF cells with flax fibers extracts followed by cells exposure to H₂O₂, resulted in elevated DNA contents in the comets heads by 21, 31 and 37% with the extracts from M50, B14 and M50+B14 flax fibers, respectively. The extract from non-transgenic Nike flax fibers exposed to H₂O₂, caused an increase of DNA content in the comets heads by 21%, when compared to relative controls (cells not incubated with the tested flax materials). Another method of assessment of the DNA strand breaks abundance in NHDF cultures incubated with the tested flax materials and then exposed to H₂O₂ were estimations of the comets tails length as given in Figure 4.

Figure 4A shows that in cultures incubated with flax fabrics a mean length of the comets’ tails (containing damaged DNA with strand breaks) was lower than in relative controls, except of the cultures carried out in the presence of small area (0.5 cm²) of Nike, M50 and B14 fabrics, in those cultures a small increase (by 10-17%) of oxidatively damaged DNA was estimated. The decrease of DNA content in the comets’ tails was especially significant in cultures incubated with M50+B14 fabric, even by 40% when compared to the control cultures. Figure 4B shows that incubation of NHDF cells with extracts from flax fibers caused decreased tails length of the comets in cultures exposed to H₂O₂. Low length of the comets’ tails was especially well-pronounced (by 28-30%) in cultures incubated with M50+B14 extract. The tested flax fabrics as well as extracts from flax fibers significantly enhanced proliferation.
rate of human skin fibroblasts (NHDF) in 48 h cultures, as was shown in histograms in Figure 5.

Figure 5A provided evidence for strong stimulatory effect of fabrics made from transgenic flax on proliferation of NHDF cells. In cultures incubated for 48 h with M50, B14 and M50+B14 fabrics, cell proliferation was augmented even by 40, 28 and 15%, respectively, when compared to the control cultures (without the flax fabrics). Whereas incubation of cell cultures in the presence of non-transgenic Nike flax fabric did not influence on NHDF cells proliferation. As is shown in Figure 5B, proliferation of NHDF fibroblasts in the presence of flax fibers extracts was also elevated even by 28, 35 and 20% in the cases of M50, B14 and M50+B14 extracts, respectively, in comparison to relative control cultures. It could be noted that extract from non-transgenic Nike flax fibers also enhanced proliferation of NHDF cells up to 28% above that in the relative control cultures. Tested flax fabrics markedly stimulated a generation cycle NHDF cells, as was estimated with flow cytometry by means of BrdU incorporation assay. Histograms presented in Figure 6 show the frequency cells in the S phase of the cell generation cycle (DNA replication phase) in NHDF cultures incubated for 48 h in the presence the tested flax fabrics.

Table 1. Multicriterial analysis of flax fabrics influence on enhancement of fibroblasts activity in the culture. The results of five in vitro tests obtained with the flax fabrics surface area of 1.0 cm² were compared according to the method described in Experimental section.

<table>
<thead>
<tr>
<th>Rating and ranking criteria</th>
<th>Tested flax fabric</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nike</td>
</tr>
<tr>
<td>Proliferation increase</td>
<td>0.15</td>
</tr>
<tr>
<td>Pace of cell cycle increase</td>
<td>0.20</td>
</tr>
<tr>
<td>Viability of fibroblasts cultures increase</td>
<td>0.15</td>
</tr>
<tr>
<td>Frequency of apoptosis decrease</td>
<td>0.20</td>
</tr>
<tr>
<td>Resistance to oxidative damage increase of the DNA content in the comets’ heads</td>
<td>0.30</td>
</tr>
<tr>
<td>Total (sum of the results in columns)</td>
<td>1.00</td>
</tr>
</tbody>
</table>
The tested flax fabrics markedly elevated cells frequency in the S-phase, and the effect was significantly stronger in the cases of fabrics from transgenic flax than in the case of non-transgenic flax. When compared to the relative control cultures (without flax fabrics) M50, B14 and M50+B14 fabrics increased frequency of NHDF cells in the S-phase of cell cycle even by 65, 75 and 60%, respectively. In the case of non-transgenic Nike flax, the fabric caused an increase of cells in the S-phase of cell cycle by about 43%. These results show that flax fabrics, especially those made from transgenic flax, strongly augment replication/proliferation of human dermal fibroblasts. Flax fabrics ability to activate human dermal fibroblasts were compared with the multicriterial analysis with the results obtained in five in vitro tests. Rating and ranking criteria of the results were selected in the aspect of their potential importance for skin cells health and for wounds repair. The multicriterial analysis was carried out with the results obtained in NHDF cultures incubated for 48 h with the surface area of 1.0 cm² and a final score of the analysis is given in Table 1.

Sums of the results obtained in the case of each fabric with five biological tests show that fabric from B14 plants exerts the most beneficial overall effect on cultures of human dermal fibroblasts. Total impact of B14 fabric on NHDF cells was almost 2x as high as that of M50+B14 fabric and almost 7x as high as that of non-transgenic Nike flax fabric. However, in the case of fabric from M50 plant, the overall stimulatory influence on fibroblast cultures was about 1.6x lower than the effect of non-transgenic Nike fabric. Content of selected compounds estimated in the tested flax fabrics are given in Table 2.

<table>
<thead>
<tr>
<th>Estimated compounds</th>
<th>Nike</th>
<th>M50</th>
<th>B14</th>
<th>M50+B14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total free phenolics</td>
<td>5.30 ± 0.3</td>
<td>3.21 ± 0.2</td>
<td>2.40 ± 0.31</td>
<td>2.37 ± 0.12</td>
</tr>
<tr>
<td>Total bound phenolics</td>
<td>28.98 ± 2.1</td>
<td>37.99 ± 3.4</td>
<td>36.51 ± 2.3</td>
<td>36.64 ± 2.7</td>
</tr>
<tr>
<td>Polyhydroxybutyrate</td>
<td>---</td>
<td>51.0 ± 2.1</td>
<td>---</td>
<td>20.0 ± 1.5</td>
</tr>
<tr>
<td>Polyamines (putrescine, spermine, spermidine)</td>
<td>10.25 ± 2.4</td>
<td>15.25 ± 5.4</td>
<td>41.04 ± 9.2</td>
<td>30.25 ± 8.5</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.065 ± 0.006</td>
<td>0.153 ± 0.008</td>
<td>0.098 ± 0.01</td>
<td>0.125 ± 0.006</td>
</tr>
<tr>
<td>Sterols (campesterol, stigmasterol, β-sitosterol)</td>
<td>498.56 ± 15.2</td>
<td>717.17 ± 14.5</td>
<td>580.28 ± 35.1</td>
<td>685.92 ± 31.2</td>
</tr>
</tbody>
</table>

The content of polyamines in the tested fabrics strongly correlated with the total beneficial effect estimated with the multicriterial analysis and the Spearman’s rank correlation coefficient \( r_s = 0.967 \) proved almost complete correlation. In contrast, phenolic content, both free and bound, lutein and sterol contents in the tested fabrics exhibited moderate correlation.

Table 2. Contents of selected group of compounds estimated in the tested flax fabrics.

Table 3. Spearman’s rank correlation coefficient \( r_s \) between the total favorable effects of the fabrics on fibroblast viability and in vitro proliferation as assessed with the multicriterial analysis (MCA) and the contents of selected group of compounds in the tested flax fabrics.

<table>
<thead>
<tr>
<th>Estimated compounds</th>
<th>Spearman’s rank correlation coefficient ( r_s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total free phenolics</td>
<td>-0.4</td>
</tr>
<tr>
<td>Total bound phenolics</td>
<td>-0.4</td>
</tr>
<tr>
<td>Polyamines</td>
<td>0.967</td>
</tr>
<tr>
<td>Lutein</td>
<td>-0.4</td>
</tr>
<tr>
<td>Sterols</td>
<td>-0.4</td>
</tr>
</tbody>
</table>

\( r_s \leq 0.2: \text{weak, } r_s \leq 0.4: \text{moderate, } r_s \leq 0.8: \text{strong, } r_s =1.0: \text{complete (according to J.P. Guilford).} \)
ate inverted correlation (-0.4) with the beneficial effects estimated in fibroblasts cultures.

**DISCUSSION**

Fabrics from genetically modified flax contain several components acting as antioxidants and anti-inflammatory compounds, as polyunsaturated fatty acids, sterols and a wide group of hydrophilic phenolic compounds (1–3). Previously, it was documented that transgenic flax fabrics significantly enhanced healing of skin wounds lesions (5, 6). Biologically active components as phenolic compounds are released to water solution when fabrics from transgenic flax are soaked in saline solution (2). Accordingly, compounds from flax fabrics are probably easily released to moist environment of skin wounds, and their interaction with cells within a wound could significantly influence on a healing process. Results presented in this paper would confirm this presumption, since extracts from flax fibers strongly activate human dermal fibroblasts in five performed biological tests. The results of the tests obtained with extracts of fibers were even better than those estimated with corresponding flax fabrics. For example, extracts from flax fibers significantly increased fibroblasts cultures viability, lowering the frequency of “spontaneous” apoptosis in NHDF cultures, whereas corresponding flax fabrics slightly increased (although not significantly) frequency of apoptotic cells, probably due to mechanical irritation of cultured cells with fabrics pieces. The results show that extracts from transgenic flax fibers would be a promising source of various flax-derived products, as solutions, creams, oils and emulsions, for improving skin cell physiology and wound healing. The impact of tested flax materials (both flax fabrics and extracts) on in vitro fibroblasts proliferation was marked by 20–40% when compared to the relative controls (cultures without flax materials). Cell cycle analysis proved that the presence of transgenic flax fabrics in cell culture strongly activated proliferation cycle of NHDF cells, and frequency of cells in the S-phase of generation cycle was elevated by 60–75%, when compared to the relative control cultures. Such strong stimulation of fibroblasts proliferation should be perceived beneficial for healing process of skin wounds. Since transgenic flax fibers contain elevated content of polyphenols and other antioxidants (1–3), cells exposed to flax derived polyphenols should be more resistant to oxidative stress. In fact, NHDF cells incubated for 48 h with flax fabrics or with extracts from flax fibers and then exposed to acute oxidative stress by addition to cell cultures of H$_2$O$_2$ [200 µM, 20 min, 3°C] displayed significantly lower level of DNA damage when compared to the controls (without incubation with the tested flax materials). The comet assay results showed both increased DNA content in the comets’ heads and lower length of the comets’ tails (containing damaged, relaxed DNA loops), which indicated decreased number of DNA strand breaks. Apparently, an exposition of human fibroblasts to the tested flax materials markedly augment cell resistance to oxidative stress.

Literature data prove that activation of fibroblasts proliferation, their differentiation into myofibroblasts, and enhanced production and release of several components acting as antioxidants and anti-inflammatory compounds, as polyunsaturated fatty acids, sterols and a wide group of hydrophilic phenolic compounds (1–3) would be a promising source of various flax-derived products, as solutions, creams, oils and emulsions, for improving skin cell physiology and wound healing. The impact of tested flax materials (both flax fabrics and extracts) on in vitro fibroblasts proliferation should be considered as valuable future component of culture media for in vitro cultures of skin substitutes, skin transplants. Compared with the multicriterial analysis total effects with five in vitro tests, proved that the fabrics from transgenic flax B14 and M50+B14 exhibited the strongest stimulation of NHDF cells, and could be recommended for further studies to elaborate new type bandage improving skin wound healing. Analysis of chemical composition of the tested fabrics showed the marked elevation of polyamine, lutein, sterols and bound phenolics contents in transgenic flax fabric compared to non transgenic flax (Nike). Particularly, considerable differences between fabrics from transgenic vs. non transgenic flax concern markedly increased content of polyamines. The content of polyamines in the tested fabrics strongly correlated with the total beneficial effect estimated with the multicriterial analysis and the Spearman’s rank correlation coefficient 0.967 proved almost complete correlation.

Natural polyamines, putrescine, spermidine, and spermine, are found in every living cell, additionally, cells could absorb polyamines from their surroundings (25). Polyamines stabilize nucleosomes and facilitate DNA condensation. They protect DNA from ionizing radiation, reactive oxygen species and are involved in regulating the expression of several genes, notably those involved in their own metabolism and in cell survival and proliferation.
(25). In conclusion, fabrics from transgenic flax type B14 and M50+B14 are the most potent activators of NHDF cells in applied in vitro tests. They could be recommended for elaboration of new type bandage to facilitate skin wound healing.

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