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Oxidative Stress Parameters of L929 Cells Cultured on Plasma-Modified PDLLA Scaffolds

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Abstract Oxidative stress may produce high level of reactive oxygen species (ROS) following cell exposure to endogenous and exogenous factors. Recent experiments implicate oxidative stress as playing an essential role in cytotoxicity of many materials. The aim of this study was to measure intracellular malondialdehyde (MDA), advanced oxidation protein product (AOPP) levels, and superoxide dismutase (SOD) activities of L929 fibroblasts cultured on PDLLA, polyethylene glycol (PEG), or ethylenediamine (EDA) grafted PDLLA by plasma polymerization method. Cell proliferation on these scaffolds was studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay. The study showed that MDA, AOPP levels, and SOD activities in L929 fibroblast cells cultured on all scaffolds were significantly different compared to the control group and each other. The highest MDA (0.42±0.76 nmol/mg protein), AOPP (14.99±4.67 nmol/mg protein) levels, and SOD activities (7.49±3.74 U/mg protein) were observed in cells cultured on non-modified scaffolds; meanwhile, the most cell proliferation was obtained in EDA-modified scaffolds (MDA 0.15±0.14 nmol/mg protein, AOPP 13.12±3.86 nmol/mg

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protein, SOD 4.82±2.64 U/mg protein). According to our finding, EDA- or PEG-modified scaffolds are potentially useful as suitable biomaterials in tissue engineering.

Keywords Biocompatibility · Oxidative stress · PDLLA scaffolds · L929 fibroblasts · MDA · SOD · AOPP

Introduction

Tissue engineering is an interdisciplinary and multidisciplinary field. It has shown a great promise in generating living alternatives for harvested tissues and organs for transplantation and reconstructive surgery. Materials and fabrication technologies are critically important for tissue engineering in designing temporary artificial extracellular matrix (scaffolds), which support three-dimensional tissue formation. The evaluation of the biological response to a material should include material safety and biocompatibility procedures [1]. In addition to the potential problem of toxic contaminants leaching out from the implant, such as residual monomers, stabilizers, emulsifiers, and many other types of additives, it is also necessary to consider the potential toxicity of the degradation products and subsequent metabolites [2]. Every material that aims to be used in biomedical applications needs to be screened for its biocompatibility. The biocompatibility of a material, while defined as the answer of cells to contact with a material or with its leachables, can be equated with the characteristics of degradation and toxicity [3]. In other words, a biocompatible material should not influence negatively the organism nor be influenced by the surrounding environment while performing a particular function [4].

PDLLA is expected to have wide applications not only as a biodegradable plastic but also as a biomedical material [5, 6] due to its excellent properties, such as mechanical strength, compatibility, transparency, safety, and adjustable hydrolyzability. As it is degradable in the human body, it is particularly suitable for the application of implants which are used only temporarily for the healing process [7].

Oxidative stress is caused by an imbalance between the oxidant and antioxidant systems in favor of the oxidants. Reactive oxygen species (ROS) can be formed by several mechanisms. These could be mitochondrial electron transport chain (ETC), nitric oxide synthase, NADPH oxidase, xanthine oxidase, cytochrome P450, and lipoxygenase/cyclo-oxygenase pathways, and the auto-oxidation of various substances, particularly catechol-amines [8]. Malondialdehyde (MDA) is a biomarker of lipid peroxidation that is closely correlated with level of oxidative stress [9]. Advanced oxidation protein products (AOPP) are the dityrosine-containing and cross-linking protein products formed during oxidative stress by reaction of plasma protein with chlorinated oxidants. Plasma AOPP concentration is closely correlating with oxidized protein. Therefore, AOPP have been considered as the markers of oxidant-mediated protein damage [10, 11].

ROS can impair the structure of cellular membrane lipids, proteins, and DNA that may cause oxidative injury by changing the normal redox status of the major cell antioxidants as superoxide dismutase. The scavenging mechanisms of the cells operate quickly to remove the excess ROS. SOD catalyzes dismutation of the superoxide anion to hydrogen peroxide and molecular oxygen [12]. L929 fibroblast cells have already been widely used to investigate oxidative stress-induced cytotoxicity [13–15].

Biochemical changes in a cell interacted with biomaterial or biomaterial extract could indicate toxicity of the material. For a material biocompatibility, ISO 10993 standards in EU indicate the toxicity/toxicity rate of biomaterials. However, these tests did not explain

any mechanism in a cell. The aim of this study was to investigate the probable oxidative stress, measure its oxidative damage levels on lipids (MDA) and proteins (AOPP), and determine the SOD activities as an antioxidant enzyme in L929 cells cultured on non-modified and EDA- or PEG-modified PDLLA scaffolds.

Materials and Methods

Preparation and Modification of PDLLA Scaffolds

PDLLA (M_W 300 kDa, Polysciences, USA) scaffolds were prepared by using freeze-drying technique [16]. Briefly 0.3 g of PDLLA was dissolved in 10 ml of chloroform (3%, *w*/*v*). The solution was then poured onto Petri dish and frozen overnight at −80 °C. Then the porous scaffold was obtained after keeping in a freeze dryer (Christ Alpha 2-4 LD) at −80 °C for 2 days and kept in a vacuum desiccator for further analysis. PDLLA scaffolds were modified by the radio frequency glow discharge (RFGD) plasma deposition technique to improve cell attachment and used PEG (M_W 300 Da, Acros, Belgium) and EDA (M_W 60.1 g/mol). Plasma modification system (Vacuum, Prague, Czech Republic) was equipped with 13.56-MHz radio frequency generator. The plasma reactor was attached with a vacuum pump for evacuation of reactor gas. The reactor was fed with the monomer tank and argon gas during the process. The scaffolds were placed onto a wooden support deployed in the middle of the electrodes with 1 cm spaces between each of species. The argon gas was passed through the reactor at 0.1 mbar pressure in order to sweep away any reactive species like oxygen and nitrogen. Subsequently, the reactor was fed with coating compounds and the glow discharge initiated at power of 35 W. The plasma process lasted for 20 min and the argon gas was passed through the chamber again to sweep away any gaseous residue. The scaffolds were kept in vacuum for 10 min for the stabilization of the modification.

Chemical Characterization of Scaffolds

Thermo Scientific K-alpha X-ray photoelectron spectrometer was used during the surface analysis of the scaffolds. The instrument has monochromated A1 K-alpha X-rays (1,486.6 eV) to strike the surface. The analyzer pass energy was 50 eV for the high-resolution core level spectra with a beam spot of 400 μm. The curve fitting of the spectra was performed with Thermo Avantage v4.41 software. A Shirley type correction was applied to the background [17].

Culture of L929 Mouse Fibroblasts on PDLLA Scaffolds

Cell cultures were conducted in sterile 6-well tissue cell culture plastic dishes in stationary conditions Dulbecco's modified Eagle's medium (Sigma Chemical Company, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Gibco, BRL), 1 mm L-glutamine, penicillin (20,000 U/ml), and streptomycin (20,000 mg/ml). PDLLA scaffolds, having 20 mm diameter and 2 mm thickness, were sterilized with 70% ethanol for 1 h and washed in sterile phosphate-buffered saline (pH 7.4). Then scaffolds were immersed in conditioning medium for 1.5 h prior to cell seeding. Fifty microliters of cell suspension (8×10^3 cell/well) was pipetted into the each scaffold. Then they were incubated in a humidified incubator (37 °C, 5% CO_2) for 1 h. Finally, 2 ml of culture medium was added to maintain the cells

[18]. The medium was replenished every 2 days for cell proliferation and oxidative stress studies.

Cell Adhesion and Proliferation Assays

Adhesion and proliferation of L929 fibroblasts on PDLLA scaffolds were analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. It was dissolved in PBS solution at concentration of 5 mg/ml and filtered through a 0.22-μm filter then stored at 4 °C. After the different culture times, medium was aspirated and scaffolds were washed twice with PBS. Nine hundred-microliter serum-free medium and 100 μl MTT solution (5 mg ml^{-1} in PBS) were added to each sample and then incubated at 37 °C for 4 h to form MTT formazan crystals. Then the medium and MTT solution were replaced by 1 ml isopropanol–HCl (absolute isopropanol containing 0.04 M HCl) to dissolve the formazan crystals. After 30 min, the absorbance at 540 nm was determined using ASYS expert plus microplate reader. Viable cell numbers on polymer scaffolds were then determined based on their absorbance [19].

Optical Microscopy Studies

Optical microscopy studies were carried out on cultured L929 fibroblasts on all PDLLA scaffolds. For optical microscopy studies, cultured cells on the scaffolds were washed with PBS, fixed in acetone/methanol (1:1) at 4 °C for 10 min, and examined after crystal violet and methylene blue stain, respectively. The PDLLA scaffolds were examined with Olympus IX51 Inverted microscope and took photographs of the cells on the scaffolds.

Oxidative Stress Parameters

After 3, 5, 7, and 10 days, cultured L929 fibroblasts were detached from scaffolds with a trypsin–EDTA solution and protected at −80 °C until the measurement of the biochemical parameters. Untreated L929 fibroblasts were used as control group. Protein contents were determined by the method of Lowry et al. using bovine serum albumin as the standard [20].

MDA Measurement

MDA levels were determined according to the Ohkawa method (1979). Fibroblasts were homogenized in cold % 1.15 KCL. One hundred fifty microliters of distilled water, 50 μl supernatant, 50 μl SDS, 375 μl TBA, and 375 μl acetic acid were mixed and heated at 95 °C for an hour. After cooling, 1,250 μl *n*-butanol/pyridine (15:1) was added to each samples and centrifuged at 4,000 rpm for 10 min. The intensity of pink/red color of the end product was determined at 532 nm. Malondialdehyde bisdiethyl acetate was used as the standard [21].

AOPP Measurement

Determination of AOPPs was based on spectrophotometric detection according to Witko-Sarsat et al. Fibroblasts were homogenized in ice-cold 20 mM Tris–HCl. Two hundred microliters of supernatant, 200 μl of chloramine T (0–100 μmol/L) for calibration, and 200 μl of PBS as blank were applied on a microtiter plate. Ten

microliters of 1.16 M potassium iodide and 20 μl of acetic acid were added to each well, and absorbance at 340 nm was measured immediately. The concentration of AOPPs was expressed in chloramine units (micromoles per liter) [11].

SOD Activity Assay

SOD (E.C. 1.15.1.1) activity assay was performed according to the method of Yi-Sun et al. Cells were homogenized in distilled water (1:10); 2.9 ml reaction mixture (40 ml of 3 mmol/l xanthine, 20 ml of 150 μmol/l Nitro blue tetrazolium (NBT), 12 ml of 400 mmol/l Na_2CO_3, and 6 ml of 1 g/L BSA), 50 μl supernatant, and 50 μl xanthine oxidase were mixed and incubated at room temperature for 20 min. After incubation, 1 ml 0.8 mM $CuCl_2$ was applied and monitored spectrophotometrically at 560 nm. One unit of SOD was defined as the amount of protein which causes a 50% inhibition of the rate of NBT reduction [22].

Statistics

Data are expressed as means ± standard deviations of a representative of each groups ($n=6$). Statistical analysis was performed using the Statistical Package for the Social Sciences version 11.5 software. Statistical comparisons were made by analysis of variance (ANOVA). Scheffe's test was used for post hoc evaluations of the differences among groups. In all statistical evaluations, $p<0.05$ was considered as statistically significant.

Results

High-resolution C1s, O1s, and N1s scans were carried out to determine carbon species on the surfaces of used material. All species contain aliphatic carbon bond at 284.7 eV due to carbon backbone of polymer. Characteristic C–O and C=O peaks were observed at 286.5 and 288.7 eV, respectively. Also O1s scans have two distinct peak at 532.8 and 533.5 eV for both O–C and O=C bindings. During N1s scan of EDA plasma-modified scaffolds, N–C bond peak was observed around 399.9 eV, whereas other two types of scaffolds do not have. The results and assignments are tabulated in Table 1.

The proliferation of L929 cells seeded onto the scaffolds was evaluated by MTT assay for 3, 5, 7, and 10 days (Fig. 1). The cell viability increased after 3 days in all groups and continued a gradual increase to day 10. There was no difference among groups at the 3rd

Table 1 Elemental analysis of the scaffolds

Element (peak)	PDLLA (at.%)	EDA-PDLLA (at.%)	PEG-PDLLA (at.%)	B.E. (eV)	Assignment
C(1s)	66.74	46.03	50.25	284.7	C–C
				286.5	C–O
				288.7	C–N
				289.0	C=O
N(1s)	0	13.52	0	399.9	N–C
O(1s)	33.26	40.46	49.75	532.8	O=C
				533.5	O–C

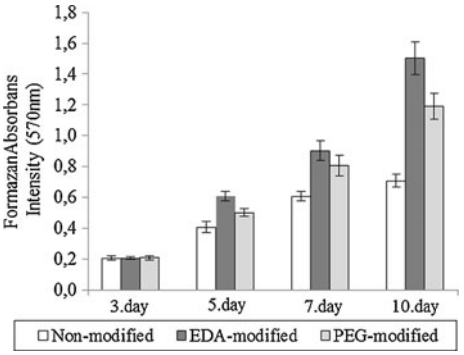

Fig. 1 Cell proliferation on PDLLA scaffolds was detected by MTT assay. Absorbance at 570 nm is represented for progressive culture times. Data are expressed as means of a representative of six similar experiments

day. However, there was significantly greater on EDA- and PEG-modified scaffolds than the non-modified after the 5th day. At the end of day 10, the lowest cell proliferation was shown on non-modified while the highest on EDA-modified scaffolds.

Morphological evaluation of L929 fibroblasts on PDLLA scaffolds was investigated by optical microscopy. Cells were seeded at a density of 2×10^5 cells/ml. After 72 h, cells were stained with crystal violet-methylene blue (A, B, C). There were no significant morphological differences between cells grown on the scaffolds and cells cultured on polystyrene (Fig. 2; magnification is 10×10).

As Fig. 3 shows, MDA levels of cells cultured on PEG-modified scaffold was statistically increased compared with control at the 3rd day. A significant difference was not observed in cells cultured on the other two scaffold types compared with control. In the same way, on the 3rd day, three groups were compared with each other, and the elevation of lipid peroxidation of cells on PEG-modified scaffold was statistically significant than the

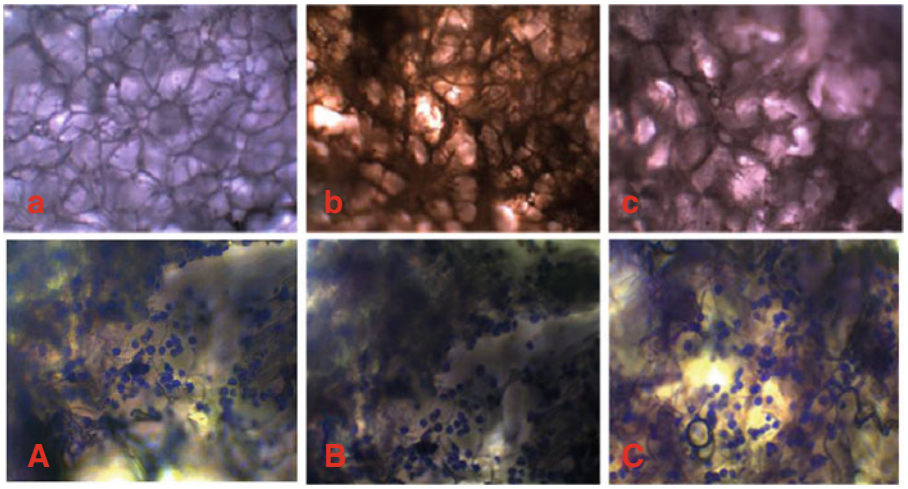

Fig. 2 Optical microscopy photographs of crystal violet and methylene blue stained L929 cell cultured on the **a** non-modified, **b** EDA-modified, and **c** PEG-modified PDLLA scaffolds at 3 days (10×10). Optical images of **a** non-modified, **b** EDA-modified, and **c** PEG-modified PDLLA scaffolds (10×20)

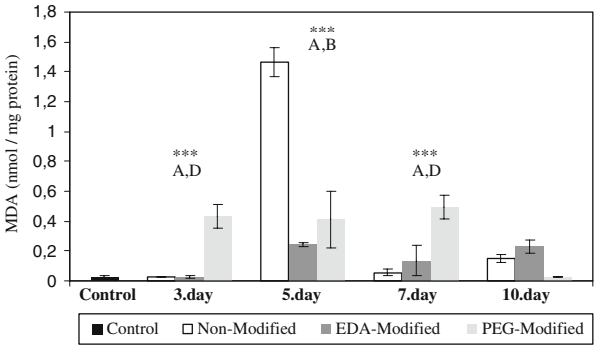

Fig. 3 MDA levels of L929 fibroblasts cultured on PDLLA, EDA-modified, or PEG-modified PDLLA scaffolds (mean ± SD). Statistical significance of ANOVA: ***$p<0.005$. Scheffe's test ($p<0.05$): **a** control, L929 cells not interacted with a scaffold; **b** non-modified PDLLA scaffold; **c** EDA-modified PDLLA scaffold; and **d** PEG-modified PDLLA scaffold

others. On the 5th day, only rapid increasing in MDA levels of L929 fibroblasts on non-modified scaffold was found significant. In spite of high MDA contents of cells cultured on EDA- or PEG-modified scaffolds, there was not a significant difference. However, low lipid peroxidation of L929 fibroblasts on EDA- or PEG-modified scaffolds was statistically significant compared with cells on non-modified scaffold. MDA levels of cells cultured on PEG-modified scaffold increased on the 7th day significantly with respect to control and cells that were cultured on the other two scaffolds at the same day. There was no significant difference observed in all cells compared with the control and each other on the 10th day. MDA levels of the cells cultured on PEG-modified started to increase on the 3rd day, continued to increase till 7th days but decreased on the 10th day significantly. According to these results, MDA levels of the cells cultured on PEG-modified scaffolds increased and then decreased earlier than the others.

AOPP levels of all groups were increased significantly on the 3rd day compared with the control group (Fig. 4). AOPP content of cells cultured on EDA-modified scaffold at that day was lower than the other types statistically. On the 5th day, increasing protein oxidation of cells seeded on only non-modified scaffold was important, and this level was at

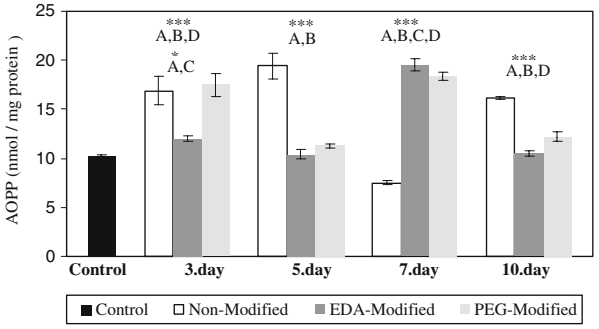

Fig. 4 AOPP levels of L929 fibroblasts cultured on PDLLA, EDA-modified, and PEG-modified PDLLA scaffolds (mean ± SD). Statistical significance of ANOVA: *$p<0.05$; ***$p<0.005$. Scheffe's test ($p<0.05$): **a** control, L929 cells not interacted with a scaffold; **b** non-modified PDLLA scaffold; **c** EDA-modified PDLLA scaffold; and **d** PEG-modified PDLLA scaffold

maximum. It was observed a significant elevation in AOPP levels on the 10th day again. AOPP contents of L929 cells cultured on EDA-modified and PEG-modified scaffolds were found parallel. Nevertheless, these levels were over the control, and protein oxidations were the highest on the 7th day. Protein oxidation of cells cultured non- and PEG-modified scaffold was significantly increased with respect to control at day 10.

Antioxidant enzyme activities in cells cultured on the 3rd, 5th, 7th, and 10th days are exhibited in Fig. 5. On the 3rd day, SOD activities increased in cells on all scaffold types. Whereas enzyme activities of cells on non-modified scaffolds increased to maximum on the 5th day; in the other two types, the maximum increase was found on the 7th day. For the 5th day, enzyme activities in all the group cells significantly differed from control and each other. At longer culture times (10th day), SOD activities of L929 on non-modified were higher than the others. Antioxidant effect in cells cultured on non-modified and PEG-modified scaffolds were statistically significant compared with control.

In the present study, we determined that MDA, AOPP levels, and SOD activities in L929 fibroblasts cultured on EDA- or PEG-modified scaffolds were always higher than the cells cultured on non-modified scaffold at day 7. A parallel undulation was observed in all cell groups seeded on non-modified scaffold considering three parameters. In other words, end products of protein and lipid breakdown in these cells synchronously increased and decreased. SOD activity of these cells showed parallel effect which increased and decreased at the same days in response to this stress. We have seen that enzyme activity in L929 fibroblasts on PEG-modified scaffold was more stable. It slowly rose up to the 7th day and then declined. As a matter of fact, not only protein but also lipid destruction in L929 fibroblasts cultured on PEG-modified scaffold at day 7 was greater than other cells with PEG-modified because of the SOD activity.

Lipid peroxidation, protein oxidation levels, and antioxidant enzyme activities in cultured cells treated with three types of scaffolds (total value of 3, 5, 7, and 10 days) were exhibited in Table 2. All of these levels were clearly higher in cells cultured on non-modified scaffold, and the levels were lower in cells cultured on EDA-modified scaffolds.

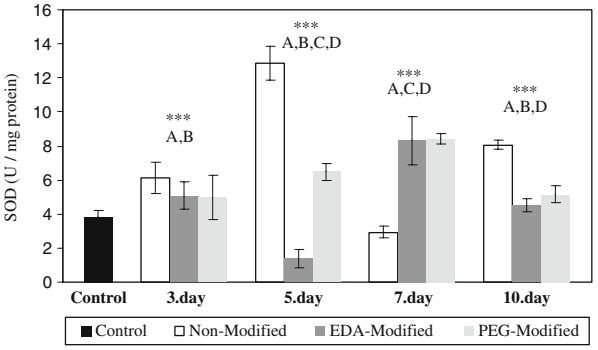

Fig. 5 SOD activities of L929 fibroblasts cultured on PDLLA, EDA-modified, and PEG-modified PDLLA scaffolds (mean ± SD). Statistical significance of ANOVA: ***$p<0.005$. Scheffe's test ($p<0.05$): **a** control, L929 cells not interacted with a scaffold; **b** non-modified PDLLA scaffold; **c** EDA-modified PDLLA scaffold; and **d** PEG-modified PDLLA scaffold

Table 2 Total average levels of biochemical parameters in cells cultured on different scaffolds

	MDA (nmol/mg protein)	AOPP (nmol/mg protein)	SOD (U/mg protein)
Non-modified	0.42±0.76	14.99±4.67	7.49±3.74
EDA-modified	0.15±0.14	13.12±3.86	4.82±2.64
PEG-modified	0.34±0.22	14.82±3.27	6.26±1.59

Discussion

In tissue engineering, bioabsorbable polymer scaffolds are used to support cells and growing tissue until they are replaced by the body's own extracellular matrix [23]. PDLLA scaffold, as a good support material and most commonly using for tissue engineering, may offer distinct advantages in its sterilizability and relative biocompatibility [24].

Different cellular aspects were analyzed in order to observe the cell viability and functions: morphology, mitochondrial function, and oxidative stress parameters. Moreover, a stimulation of mitochondrial redox activity produced by the contact with a polymer was observed [25]. Exposure of cells to various compounds may interfere with their living systems and result in cytotoxic effect [26]. The aim of this study was to investigate the interaction of L929 fibroblasts with modified and non-modified PDLLA scaffolds inducing oxidative stress in cells. These cells are recommended as reference cell line for the cell culture tests of biomaterials.

In our study, PDLLA scaffolds were modified with PEG or EDA by the RFGD plasma deposition technique before cell culture in order to improve cell adhesion and proliferation. These modifications were plentiful concluded. EDA and PEG modification of the PDLLA scaffolds affected positively cell attachment and proliferation. Cell proliferation on EDA-modified scaffolds was the highest value at different days according to our MTT results while the lowest was on non-modified. In addition, the morphology of the cells was similar, after 3rd, 5th, and 7th days of L929 cell cultures on EDA- and PEG-modified.

There is now growing evidence that the reactive oxygen species affect on cell proliferation and affect the antioxidative status of various cell types [27]. Some studies showed that alteration of viability in cell cultures is associated with a significant enhancement of oxidative processes [28, 29]. In these documents, it has not been shown that an increase in ROS formation is implicated in cell damage and cytotoxicity. An alternative approach to clarify the mechanisms for oxidative cellular damage by ROS is to quantify stable end products of ROS reactions with macromolecules [30].

We investigated the probable ROS production and cellular oxidative stress in the interaction of L929 fibroblasts with non-modified and EDA- or PEG-modified PDLLA scaffolds. For this reason, we measured the oxidative damage on lipid and protein due to detected MDA and AOPP levels, respectively, and SOD activity as a cell defense system. Cell oxidative stress is a quite new and interesting topic in tissue engineering research, and not many studies have been performed in order to evaluate oxidative stress parameters related to the effects of the scaffolds treatment [31].

Serrano et al. showed that a stimulation of mitochondrial redox activity is produced by the contact with poly(ε-caprolactone) (PCL) film [25] which PCL is a biodegradable and biocompatible polymer. In this study, L929 fibroblasts cultured on PCL films showed an increase of mitochondrial redox activity parameter after 24 h (176%) referred to control cells (100%). This effect was decreasing during 4 days and disappeared at longer culture times (on the 7th day). High mitochondrial redox activity causes electron leakage and

radical and oxidant production. According to our data, as markers of oxidative stress, the highest MDA and AOPP content of L929 fibroblasts cultured on non-modified scaffolds was observed at 5th day because lipid peroxidation and protein oxidation have occurred in this group cells at most. Actually, it is found that the maximum lipid peroxidation was constituted at the 5th day in the cultured cells. Considerable and significant rise in these biomarkers provide direct evidence that scaffold exposure is a relevant source of oxidative stress in which local production of oxidative radicals plays important roles.

The cell's permeability is associated with their membrane stability. Alteration of membrane phospholipids through lipid peroxidation by oxidants that other investigators have reported as MDA elevation could cause a loss of membrane stability and integrity leading to increase trans-membrane permeability [26, 32]. Some studies have revealed that mitochondrial ETC is the most important source of ROS in mammalian cells [33], and alterations in mitochondrial energy metabolism may be directly associated with oxidative stress [34] and high MDA and AOPP levels. MDA levels of the cell cultured on PEG-modified scaffolds were increased and then decreased earlier than the others. On the other hands, PEG-modified scaffolds caused lipid peroxidation in the cells earlier than the other scaffolds, but these cells more quickly recovered than the other cultured cells. We also determined the most lipid destroying happened at day 5 while protein destruction was at the 7th day cells cultured on EDA-modified scaffold.

Some results indicate the possible relation of oxidative stress with cell adhesion [31, 35, 36]. It has observed that ROS content of L929 fibroblasts cultured on PCL films has similar to control after 24 h. However, in cells cultured on PCL, this parameter has increased after 4 days in culture. After 7 days, ROS content of cells has decreased [31]. Our findings were in agreement with this study. Lipid peroxidation and protein oxidation were higher at day 5 in cultured cells on non-modified scaffold and at day 7 in cultured cells on PEG-modified scaffold. All cell cultures exhibited high level of AOPP at the 3rd day. It may be started by protein and lipid destruction and oxidative stress parameters produced by the interaction of cells with scaffold surfaces. It was important to compare with the oxidative stress the parameters of L929 cells cultured on three different scaffolds and investigate which scaffold has minimum cytotoxicity. So according to general average, cells cultured on EDA-modified scaffold were exposed to the least stress because the lowest lipid and protein destructions in these cells occurred.

For the cells, elaborate antioxidant systems are necessary to avoid the oxidative damage [26]. The reduction of molecular O_2 by the mitochondrial ETC is essential for generating the most biological energy. An electron is gained by molecular O_2, yielding superoxide ($O_2^{\bullet-}$). The scavenging mechanisms of the cells operate quickly to remove the excess ROS. SOD catalyzes dismutation of the superoxide anion to hydrogen peroxide and molecular oxygen [12]. In this study, it is realized that the SOD activities were high at 3rd day in all group cells compared to the control. Soheili et al. [37] found that dental restorative biomaterials induce intracellular GSH depletion in human gingival fibroblasts at 24 h, being indicative of a pro-oxidant effect. Other investigators also suggested a slow depletion of GSH stores in cells on PCL at short times (1 day) and increase of GSH content of cells culture on PCL films after 4 days [31]. We considered that radical scavenger superoxide dismutase has increased because of the radical production at day 3. It was not surprising that the SOD activities of cells cultured on non-modified scaffold were at maximum level at 5th day which was the highest destroy productions of lipid and protein. SOD activity of fibroblasts on non-modified scaffold contributed the level

decreases of MDA and AOPP at day 7. Increase of MDA and AOPP levels in cells cultured on PEG-modified scaffold triggered SOD activity to rise in order to show its antioxidant effect at 7th day. After that, SOD has decreased the MDA and AOPP levels until it has exhausted.

In conclusion, the interaction between the L929 fibroblasts and non-modified and EDA- or PEG-modified PDLLA scaffolds induced the oxidative stress significantly. Average value of antioxidant enzyme activity in the cells cultured on non-modified scaffold (SOD 7.49±3.74 U/mg protein) was found at maximum. It was parallel to high levels of lipid peroxidation (MDA 0.42±0.76 nmol/mg protein) and protein oxidation (AOPP 14.99±4.67 nmol/mg protein; $p<0.005$). Therefore, it was markedly more less cells proliferation on these scaffolds. On the contrary, EDA-modified scaffold caused the lowest stress (MDA 0.15±0.14 nmol/mg protein, AOPP 13.12±3.86 nmol/mg protein) and so the lowest SOD activity (4.82±2.64 U/mg protein) in the cells. In other words, EDA-modified scaffold is more biocompatible than the others. That is to say that non-modified scaffold was more cytotoxic than the others. The radical formation might have occurred by cytotoxic effect of scaffold through oxidative damage of lipid and protein. Actually, cell proliferation on EDA-modified scaffold was at most. SOD activity provided an equilibrium, and so it was observed cell proliferation in spite of oxidative stress. Our preliminary results suggest that PEG- or EDA-modified scaffolds are better materials for supporting L929 cell growth, and these scaffolds might be used in tissue engineering studies.

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