OLIVE LEAF EXTRACT LOADED SILK FIBROIN/HYALURONIC ACID NANOFIBER WEBS FOR WOUND DRESSING APPLICATIONS

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In this study a novel wound contact layer was developed by entrapping polyphenols obtained from olive leaf extract (OLE) inside the nanofibers made from the blend of hyaluronic acid and silk fibroin, produced by co axial electrospinning method. OLE has powerful antibacterial, anti fungal and antiviral properties and also known as a good antioxidant. The purpose of coaxial electrospinning was to achieve the controlled release of OLE by capturing it inside nanofibers. TEM images confirmed that OLE was successfully captured. Release profile of OLE loaded nanofibers indicated an initial burst release followed by a more gradual release.

In vitro cytotoxicity tests indicated that the developed electrospun coaxial nanofibers were non-toxic.

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1. Introduction

The history of wound dressings goes back to ancient times. At the beginning, wound dressings were used only to prevent further damage by protecting the wound from the external environment, but today modern wound dressings also provide an environment for an optimal healing process [1]. An ideal wound dressing should provide a moist healing environment [2], allow exchange of O2 and CO2, act as a barrier to microorganisms, promote epithelial restoration, remove excess exudates to minimize infection [3,4], and be biocompatible. It should also have low adherence and can be easily removed without disturbing newly occurring tissue layers [4,5]. Another desirable feature is to include compounds which accelerate the healing. For example, with the addition of silver metal ions or salts, antibiotics and antibacterial components such as honey to this layer, the wound dressing attains antimicrobial characteristics and the growth of infection-causing bacteria is prevented to a large extent [6,7]. The previous studies revealed that in addition to antimicrobial compounds, the use of antioxidant compounds in wound dressings speeds up the healing of chronic wounds [8,9,10,11]. Antioxidants can be defined as the active compounds which have some impact mechanisms to reduce or delay the harmful effects of free radicals [12]. Actually, living systems have their own cellular defense mechanisms for free radicals. However, some external factors (air pollution, radiation, chemicals, physical stress, tissue damages due to injury, etc.) and some internal factors (some enzymes and immune system products) increase the occurrence and the cellular concentration of free radicals. It has been known for years that several plant extracts which contain natural compounds with different biologic activities such as antioxidant, antimicrobial and anti-tumoral activities have wound healing

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effect[13]. Olive leaf extract, which is one of them, has been used traditionally in wound healing and acne treatment. It is believed that wound healing effect of olive leaf extract is due to oleanolic acid and flavanoids in the extract. In addition, some active compounds in olive leaf extract have positive effect on fibroblast, which is necessary for healthy skin and they have also perfect antioxidant activities particularly in oxidative stress situations[14]. Although active compounds which help wound healing can be directly applied to the wound, for the most effective results they should be transfer to the wound in a specific amount over a specific time period (controlled release). This is only possible with the development of functional wound contact layers for wound dressings. Materials in different forms (woven, nonwoven, film, etc.) can be used as wound contact layer, but a surface made of nanofibers offers several advantages. The high surface area provided by nanofiber matrix allows oxygen permeability and prevent fluid accumulation. On the other hand, small pore size doesn’t let micro-organisms pass through the wound contact layer [15]. For nanofiber production the most advantageous and simple method is electrospinning method. This method consists of nano-scale fiber spinning from either a polymer solution or molten polymer by means of electrostatic forces. In this technique, it is important to choice a suitable polymer that enhances wound healing process. A nanofiber matrix produced from a biopolymer that allows cell adhesion will help the propagation of epithelial cells from the wound border by functioning as a scaffold. Silk fibroin and hyaluronic acid are two such biopolymers. Previous studies have showed that they both help cell migration [16-21]. Silk fibroin (SF) has high mechanical strength, good biocompatibility, good oxygen and water vapor permeability, good biodegradability, and causes minimal inflammatory response [22, 23, 24]. It supports collagen synthesis and epitalization[17]. Hyaluronic acid (HA) is a linear polysaccharide discovered in bovine vitreous humour by Meyer and Palmer in 1934. It is an important constituent of the extracellular matrix of many soft tissues in the body [25].

SF based nanofibers can be produced rather easily by electrospinning, but it is difficult to produce HA based nanofibers due to its high viscosity and high surface tension. In order to overcome this problem various systems such as using a combination of air flow and electrospinning [26] and mixing HA with the other polymers including collagen[27], gelatin [28], and zein [29] were used previously. In this study HA was blended with silk fibroin. This study investigates the preparation and characterization of olive leaf extract (OLE) loaded coaxial nanofibers made from the blend of silk fibroin(SF) and hyaluronic (HA) acid. The morphologies of the fabricated nanofibers are examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Drug release profile of nanofibers are examined via in vitro tests. The potential use of nanofibers in wound dressing applications is evaluated through a HS2 cell culture test.

2. Experimental

2.1 Materials

Raw Silk from Bombyx mori were was obtained from the Bursa Institute for Silkworm Research (Turkey). Hyaluronic acid was purchased from Fluka-BioChemica (Switzerland). Olive leaf extract was kindly provided by DUAG Ltd. (Turkey). Polyethylene oxide (M_w = 900,000 Da) was purchased from Sigma Aldrich (USA). Formic Acid (%98) was purchased from Merck (Germany). Materials used in cell culture studies was purchased from Biochrom (Germany).

2.2 Preparation of regenerated silk fibroin aqueous solution

Raw Bombyx mori silk was treated with boiling aqueous Na_2CO_3 (0.5 wt%) solution at a bath ratio of 1:50 (w/v) for 30 min. This treatment was repeated three times. Afterward, silk was rinsed thoroughly in distilled water to remove sericin and left for drying at room temperature. The degummed silk was dissolved in a mixed triad solvent of CaCl_2·2H_2O/H_2O/EtOH with a 1:8:2 molar ratio at 78°C and 125 rpm stirring speed for about 2 hours. Then the solution was dialyzed using cellulose tubular membranes in distilled water for minimum 3 days at ~ 4°C. Dialyzed solution were put in petri dishes and dried under vacuum to obtain silk fibroin films used in the preparation of electrospinning solution.
2.3 Fabrication of OLE loaded nanofibers

Two syringe pumps (New Era Pump Systems, USA) and a high voltage power supply (Iseg Spezialelektronik GmbH, Germany) were used for electrospinning. The coaxial spinneret was consisted of a 24 gauge inner needle mounted on a 17-gauge outer needle. Coaxial electrospinning was performed at a core flow rate of 2 μL/min and at a shell flow rate of 6 μL/min. The applied voltage (V) was maintained at 20.46 kV and the distance from the spinneret to collector (rotating drum) was fixed to be 10 cm.

For shell solution the silk fibroin solution of 10% (w/v) and HA solution of 2% (w/v) in 98% formic acid were prepared separately, then SF/HA were mixed at a weight ratio of 80/20. Core solution was prepared by first dissolving polyethylene oxide in 98% formic acid at a concentration of 1% (w/v) and then adding 30mg/ml or 120 mg/ml olive leaf extract into the solution.

2.4 Characterization

The morphology of the electrospun nanofibers was observed by using Scanning electron microscopy (SEM, Philips XL 30S FEG ) at a accelerating voltage of 5 kV. Before the observation, the samples were gold sputter-coated under an argon atmosphere to make them electrically conductive. The average fiber diameter and diameter distribution of the electrospun fibers were analyzed from the SEM images by using Image J analysis software (National Institutes of Health, MD, USA).

The core–shell structure of the nanofiber were characterized by transmission electron microscopy (TEM, FEI Tecnai Biotuin G2 Spirit) at 80 kV. To obtain contrast between shell and core morphology, Bromophenol Blue (Sigma-Aldrich) was added to core solution with a ratio of 5%. Electrospun nanofibers were collected on a Holey carbon film coated 200 mesh copper TEM grid (Electron Microscopy Sciences).

2.5 In vitro drug release tests

To determine the release kinetics of the olive leaf extract from the nanofiber webs, olive leaf extract of different concentrations (1.25, 2.5, 5, 12.5, 25, 50 (μg mL$^{-1}$)) in distilled water were prepared and their absorbance at 280 nm were determined by UV spectroscopy to obtain the calibration curve $C =12.998A$ (R=0.9975)where $C$ is the concentration of OLE (μg mL$^{-1}$) and A is the solution absorbance at 280 nm.

In vitro release test method was used to reveal the release profile of nanofiber mats. In this method, 10 mg (approximately 2cmx2cm) of nanofiber samples were placed into 12 well plate and 5 mL of PBS solution was added to each well. As a negative control 10 mg of nanofiber web without olive leaf extract was used. All the samples for release experiments were incubated at 37°C for a period of 30 days. The experiment was done in triplicate. At selected time intervals (0, 2, 4, 6, 24, 48, 72 hours; 1, 2, 3 weeks), a 1-mL solution was taken out from the wells and equal volume of fresh PBS buffer was replenished. At the end of 1 month the test was finalized and the release kinetics of samples were evaluated using a spectrophotometer (Perkin Elmer,USA) by observing the characteristic absorption peak at 280 nm for olive leaf extract.

2.5.1 Total phenol content

Total phenol content of the samples taken during specific time intervals was determined by the Folin-Ciocalteu assay. 100 μL of Folin–Ciocalteu reagent (diluted 10 times with water) was added to 20 μL of sample. After 2.5 minutes to stop reaction between sample and reagent, 80 μL of 7% Na2CO3 was added, and samples were left for 1 hour at room temperature in the dark. Then absorbance values were measured by a Spectrophotometer at 725 nm. The result calculated using a gallic acid calibration curve. The total phenolic content expressed in μg of gallic acid equivalents (μg GAE/mL of extract).

2.5.2 Trolox equivalent antioxidant capacity (TEAC)

Antioxidant activity of electrospun nanofibers was determined by Trolox equivalent antioxidant capacity assay (TEAC) using the radical cation ABTS+. 2 ml of the ABTS+ stock
solution diluted with ethanol to give an absorption of $0.70 \pm 0.03$ at $\lambda = 734$ nm was added to 10 µl of the release medium. A calibration curve for the TEAC was built by using different Trolox concentrations. To calculate the TEAC of the release medium, the area under the curve derived from plotting the percentage inhibition of the absorbance as a function of time was compared with the area under the curve for Trolox standard. The TEAC was expressed as µmol of Trolox equivalents per gram sample.

### 2.5.3 HPLC analysis of phenolic compounds

HPLC analysis was carried out to detect the bioactive compounds released from the OLE loaded nanofibers during 1 month period. The HPLC equipment used was a Hewlett-Packard Series HP 1100 equipped with a diode array detector. The stationary phase was a C18 LiChrospher 100 analytical column ($4 \times 250$ mm, 5 µm particle size) thermostated at 30 °C with a flow rate of 1 ml min $^{-1}$ and the mobile phases was composed of solvent A (2.5:97.5 acetic acid/water) and solvent B (acetonitrile). The absorbance changes were monitored at 280 nm. All samples were filtered through a 0.45 µm syringe filter before HPLC analysis. Phenolic compounds in release medium was determined by comparing the retention time of compounds with standards.

### 3. In vitro cytotoxicity tests

#### 3.1 Methylthiazolylidiphenyl-tetrazolium bromide (MTT) assay

The cytotoxicity of the electrospun nanofiber’s membranes was evaluated based on a procedure adapted from the ISO 10993-12 standard test method. Three nanofiber samples: sample 1 with 3% olive leaf extract, sample 2 with 12% olive leaf extract and sample 3 without extract were prepared for MTT assay. Nanofiber samples were cut into dimensions of 30 mm x 10 mm, sterilized under UV radiation for 30 min before extract preparation. The samples were then incubated in 1 mL of DMEM at 37 °C for 48 h. After that, the nanofiber samples were removed and the extracts were obtained. For these extracts, extraction ratio was designated as 1/1 extract. Less concentrated extracts were also prepared by diluting the 1/1 extract into 1/2, 1/4 and 1/8 extract solutions.

HS2 human epidermal keratinocytes cell lines were used in cell culture study. Keratinocytes cells were cultured in DMEM HAM’s F12 supplemented with 10% fetal bovine serum % 1 L-Glutamine %0,1 penicillin/streptomycin in an incubator under a controlled atmosphere of 5% CO2 at 37 °C. When the cells reached 80% confluence, they were trypsinized with 0.25% trypsin containing 1 mL Ethylene Diamine Tetracetic Acid (EDTA) and suspended in the culture medium. Then cells were seeded into 96-well cell culture plates at a density of 1x105 cell/mL and incubated for another 24 hours at 37 C, 5% CO2. After 24 h the culture medium was replaced with serial dilutions of extraction medium. Culture medium without extract served as the negative control.

The cytotoxicity of the extracts was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) assay. Approximately, 100µL of MTT solution was added to each well. After incubation at 37°C for 24 h, 100 µL of dimethylsulfoxide was added to dissolve the purple formazan crystals. The optical density of the formazan solution was detected by a plate reader (VersaMax; Molecular Devices, Sunnyvale, CA, USA) at wavelengths of 570 and 690 nm. As negative control cells were seeded to a fresh culture medium. The percentage of cell viability was calculated as the following formula: cell viability (%)=(OD of extraction medium-treated sample/OD of untreated sample)×100. Cell viability data was expressed in the bar graphs as mean ± Standard deviation.

#### 3.1.1 Agar overlay method

HS2 human epidermal keratinocytes cells were grown in a 6-well culture plate and after 80-85% confluence the culture medium was replaced with 2 ml growth medium (agar plus nutrients) to generate the protective agar layer. To prepare growth medium 1% low melting agar solution was autoclaved at 105°C for 20 min. When agar temperature fell down to 50-60 °C, it was mixed with liquid nutrient medium (DMEM, %5 FBS, %1 NaHCO3) to get a 50/50mix and left
for drying. After solidification, nanofiber samples were carefully placed on agar. Following 4 day incubation agar was removed and cell layer was washed with PBS, giemsa stained and photographed by an inverted microscope.

4. Results and discussion

4.1 Morphology of nanofiberwebs

SEM image of nanofiber sample with 3% olive leaf extract is given in Figure 2. The fibers had an average diameter of 123.25±44.61 nm. TEM images of SF-HA / PEO-OLE nanofibers clearly shows a core–shell structure (Figure 3).

![SEM image of nanofiber sample with 3% olive leaf extract](image1)

**Fig. 2.** (a) SEM image of nanofiber sample with 3% olive leaf extract, (b) the distribution of fiber diameter

![TEM images of coaxial nanofibers](image2)

**Fig. 3.** TEM images of coaxial nanofibers

From Fig. 3(b), it could also be seen that in some parts there is a non-uniform distribution of core.

4.2 In vitro drug release tests

4.2.1 Total phenol content

Table 1 displays the weight percentage of compounds in polymer solutions. Theoretically, 10 mg sample with 3% olive leaf extract contained 1.452 mg extract and the 10 mg sample with 12% olive leaf extract contained 4.054 mg extract.
Table 1 Weight percentage of compounds in polymer solutions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shell</th>
<th>Core</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>SF</td>
<td>HA</td>
</tr>
<tr>
<td>3 % OLE</td>
<td>64.51</td>
<td>16.13</td>
</tr>
<tr>
<td>12 % OLE</td>
<td>44.86</td>
<td>11.22</td>
</tr>
<tr>
<td>Control</td>
<td>75.47</td>
<td>18.87</td>
</tr>
</tbody>
</table>

Primarily, the total phenol content of OLE used in the experiments was determined as 14.04±0.83 mg GAE/ g of OLE. To explain the mechanism of release it is important to know where the loaded extract is located in the coaxial nanofiber structure. Prior to release to find out the total phenolic content of coaxial nanofibers in terms of gallic acid equivalents (GAE) nanofiber samples were crashed by a mini bead beater (Biospec Products). Samples were dried at 30°C for 24 h in a incubator and crashed manually. A 20 μg sample with 1ml PBS was put into a 2 ml screw cap microvial and crashing was carried out by 0.1 g Zirkonyum-Silica (diameter: 0.1 mm) beads at 2500 oscillation/min. Total phenol content of medium was evaluated by taking small samples in every three minutes till no phenolic content was detected in the solution.

Total phenol analysis performed after bead beater revealed that initial release of OLE was high. This could be explained by OLE attached weakly to nanofiber surfaces or hydrophilic polymer or polymer groups inside the coaxial nanofiber structure. Hydrophilic compounds tend to gather onto the nanofiber surface[30]. Following the initial release OLE extract captured inside the coaxial structure was release as a result of crashing. Cumulative values showed that while total phenol content of the nanofibers with 12 % OLE was 1.51 mg GAE/g nanofiber, that of the nanofibers with 3 % OLE was 0.38 mg GAE/ g nanofiber.

Total cumulative phenol content released through in vitro release study was compared with the total phenol content obtained through bead beater test and how much of the OLE captured inside the structure which was released during 1 month period was determined. Results showed that at end of 1 month period 70.15 of OLE loaded to nanofibers with 12 % OLE and 69.85% of OLE loaded to nanofibers with 3 % OLE was released. Figure 4 gives the release profile of the nanofibers in terms of total phenol content. In all cases, an initial burst release was followed by a more gradual release of OLE from the nanofibers.

The shell of nanofibers made from blend of hyaluronic acid and silk fibroin. The core part include polyethylene oxide and OLE. Both hyaluronic acid and polyethylene oxide has hydrophilic characteristic. Silk fibroin has both hydrophilic and hydrophobic side groups. It is likely that the burst release at the beginning of release test due to the weak bonds between hydrophilic polymers or groups and OLE. Subsequent slow and sustained release could be associated with stronger bonds such as covalent bonds between OLE and the coaxial nanofiber structure, and the slow wetting of hydrophobic groups and finally degradation of the shell.

Fig. 4. Release profile of the nanofibers loaded with 3 and 12 % OLE in terms of total phenol content
4.2.2 Trolox equivalent antioxidant capacity (TEAC)

Initially, trolox equivalent total antioxidant capacity of OLE used in the experiments were determined as 11.62±1.22 mM TEAK/g olive leaf extract. As in the determination of total phenol content, antioxidant capacities of coaxial nanofibers were found out after the crashing with bead beater process. Trolox equivalent total antioxidant capacity of 12 and 3% OLE loaded nanofibers and control nanofibers dependent on decomposition time were found negative. It is believed that this is due to the interaction between ABTS+ radical cation and OLE. That’s why results were given as the difference between trolox equivalent total antioxidant capacity of samples and control. Trolox equivalent total antioxidant capacity of 12 % and 3 % OLE loaded nanofibers were found to be 6.28 and 3.60 mM ΔTEAK/g nanofiber, respectively. Similar to total phenol content, total cumulative antioxidant capacity released through in vitro release study was compared with the total antioxidant capacity obtained through bead beater test and how much of the OLE captured inside the structure which was released during 1 month period was determined. Release profile of compounds with antioxidant capacity showed similarity with the release profile of total phenol content. During the first 24 hours a sudden release was followed by continuous and slow release. At the end of 1 month, nanofibers loaded with 12 % OLE released 99.5 % of their total antioxidant capacity; nanofibers loaded with 3 % OLE on the other hand, released 76.6 % of their antioxidant capacity (Fig. 5).

Fig. 5. Release profile of the nanofibers loaded with 3 and 12 % OLE in terms of antioxidant capacity

Oxygen plays an important role in the wound healing process by killing bacteria, enhancing collagen synthesis, and epithelialisation. However, overproduction of reactive oxygen species causes oxidative stress and impairs wound healing. Thus, elimination of reactive oxygen species could speed up healing process[31]. Antioxidants are known to help controlling oxidative stress. That’s why we expect that OLE loaded nanofibers will promote healing process.

4.3 HPLC Analysis

For the identification and quantization of compounds of olive leaf extract released to the buffer solution during in vitro release test, high performance liquid chromatography (HPLC) analysis was conducted. Initially OLE extract was weighed and dissolved in PBS to give 1 mg/ml concentration and analyzed by HPLC. Released medium obtained through in vitro release test of 12 and 3 % OLE loaded nanofibers were solidified by a centrifugal evaporator and solid remains was solved in mobile phase and analyzed. Figure 6 shows the chromatograms of 0.1 % OLE in PBS and released mediums of nanofibers loaded with 3 and 12% of OLE. Table 2 summarizes the quantity of phenolic compounds in the release mediums.
The peaks observed in both released mediums of nanofibers loaded with 3 and 12\% OLE at early timepoints are apparent silk fibroin peaks. The main components of olive leaf extract is oleuropein. It is followed by hydroxytyrosol, a degradation product of oleuropein [32,33]. The peak around 5.8th minute belongs to hydroxytyrosol and the peak around 7.7th minute belongs to Tyrosol, which is also a degradation product of oleuropein.

As seen in Fig. 6, unlike pure OLE these degradation products were more dominant than oleuropein in the release medium obtained at the end of the in vitro release test. This can be attributed to formic acid used as solvent during nanofiber production which likely caused degradation of oleuropein and turned it into polar compounds. In 3\% OLE loaded nanofibers some of the compounds of 12\% OLE loaded nanofibers weren’t came across. It seems that those compounds were under the minimum detection limit. Oleuropein hydrolyses to form tyrosol derivatives under acidic conditions. In fact, these tyrosol derivatives are more effective than oleuropein in terms of antioxidant capacity and antimicrobial activity[32]. Another study showed that the antimicrobial effect of the combination of all of phenolic compounds from OLE was more effective than pure oleuropein, and in terms of antioxidant capacity phenolic compounds followed this order: cafeic acid, phenolic blend, rutin, oleuropein and vanillin [34]. Thus the degradation of OLE into phenolic compounds during electrospinning can be advantageous in terms of antimicrobial and antioxidative activity.
Table 2 Quantity of phenolic compounds

<table>
<thead>
<tr>
<th>OLE compounds</th>
<th>Minute</th>
<th>Peak area (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>12 % OLE loaded nanofiber</td>
<td>3 % OLE loaded nanofiber</td>
</tr>
<tr>
<td>hydroxytyrosol</td>
<td>5.80</td>
<td>8.94</td>
</tr>
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<td>tyrosol</td>
<td>7.75</td>
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<tr>
<td>catechin</td>
<td>11.21</td>
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<tr>
<td>caffeic acid</td>
<td>13.43</td>
<td>6.14</td>
</tr>
<tr>
<td>Vanillin</td>
<td>14.37</td>
<td>3.21</td>
</tr>
<tr>
<td>Rutin</td>
<td>17.57</td>
<td>8.88</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>22.22</td>
<td>5.27</td>
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</table>

4.4 In vitro cytotoxicity test

Two methods were utilized to evaluate the level of cytotoxicity of electrospun nanofibers. The first method was based on a procedure adapted from the ISO 10993-12 standard test method of indirect MTT cytotoxicity assay. In the MTT-test undiluted and serial diluted extract of test specimens were used. Culture medium without extract served as the negative control. Fig 7 show the viability of HS2 human epidermal keratinocytes cells that were cultured after 24 h incubation period. The control group (untreated cells) was taken as 100% viable. In HS2 cells, nanofibers with 12 % OLE content produced the highest cell viability results. The addition of 12% OLE inside the nanofiber structure actually improve the cell viability. Only the undiluted extract of 3 % OLE contained nanofibers exhibited a very low level of toxicity. In general, none of the samples (undiluted and diluted) had obvious cytotoxic effect on HS2 human epidermal keratinocytes cells after 24 h’s of incubation, suggesting that the developed nanofiber webs were non-cytotoxic and suitable for the further applications.

Fig.7. MTT viability assay of Keratinocytes cells seeded on the undiluted and serial diluted extract of specimens a) nanofibers without OLE; b) 3% OLE extract loaded nanofibers; c) 12%OLE extract loaded nanofibers
The second method was the agar overlay test, where specimens were separated from the cells by an agar layer. Inverted microscope images of cells are given in Fig.8. The density of HS2 cells in the cell culture plates including nanofiber webs did not differ from the control (without the nanofiber web). These results were consistent with the MTT test and further confirmed the non-cytotoxicity of the OLE extract loaded nanofiber webs.

![Inverted microscope images of HS2 human epidermal keratinocytes cells after giemsa staining](image)

**Fig. 8.** Inverted microscope images of HS2 human epidermal keratinocytes cells after giemsa staining a) Control; b) 3% OLE extract loaded nanofibers; c) 12% OLE extract loaded nanofibers d) nanofibers without OLE

**5. Conclusion**

In this study a novel wound contact layer was developed by entrapping polyphenols from olive leaf extract inside a shell made from hyaluronic acid and silk fibroin. The morphology of the resulting composite nanofibers were characterized through SEM and TEM. SEM images showed a bead-free and smooth fiber morphology. TEM images confirmed coaxial structure. Results of in vitro release experiments suggested that the silk fibroin/hyaluronic acid nanofibers were capable of releasing the OLE in somewhat controlled fashion. The initial burst release was followed by prolonged release up to 30 Days. The cytotoxicity of the OLE loaded nanofiber webs was evaluated in vitro against the human epidermal keratinocytes cell. There was no evidence of cytotoxicity.

In conclusion olive leaf extract loaded nanofiber webs developed in this study are good candidates for wound dressing applications as well as tissue engineering.

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**References**