# PROMOTE THE BIOCOMPATIBILITY OF ELECTROSPUN POLYMERIC NANOFIBERS THROUGH HERBAL EXTRACTS

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## Avstract.

"Tissue engineering" is an artificial production of biological structures in which cells are cultivated on a carrier material (scaffold). This scaffold has a decisive influence on the success of colonization with cells, that is on their growth as well as on their multiplication and tissue formation ability. The polymeric nanofibers obtained by the electrospinning method are particularly well suited to the challenges of scaffolding.We have developed the hypothesis that the treatment of these polymeric fibers with plant extracts will result in the optimization of the properties of polymer scaffold. Based on this hypothesis, we investigated the differences in cell adhesion and cell growth of epithelial cells on electrospun polymeric nanofibers when incubated with extract from the medicinal plant *Haberlea rhopdopensis*.The results of the experiments we have performed may serve as a basis for the production of improved scaffolds whose biocompatibility has been optimized by the plant extract of *Haberlea rhopdopensis*.

## Introduction

The functioning of cells, tissues or organs in the human body can be significantly and irreversibly damaged by various types of diseases. The goal of Regenerative Medicine is to provide a replacement for the sick so they can live a normal life. This was previously only possible through organ donation. However, "tissue engineering" has opened new horizons for medicine. "Tissue engineering" is the cultivation of living tissue on a carrier material so that finally a restoration of the damaged organs is achieved.

Regenerative medicine creates hope for those whose tissues or organs have been damaged in any way. That fascinated me deeply and motivated me to explore the essence of tissue engineering. As a result, we found out that the cells needed a certain base, the so-called scaffold, for their growth. The polymeric nanofibers have such properties that they are particularly well suited for the role of a scaffold. This has brought us to the question of whether we can improve their properties, so that the cells attach better to the material and can multiply faster. Our suggest that the treatment of polymeric fibers with the plant extracts of *Haberlea rhodopensis* may affect the behavior of cells in terms of their growth, proliferation and tissue formation. The endemic plant *Haberlea rhodopensis* is rich in polyphenols and other active ingredients that are responsible for their antioxidant and regenerative properties. Many research centers have devoted themselves to exploring this species (1,2,3).

## Materials and methods

Electrospinning (ES) is a process for producing polymeric nanofibers that has been known for 70 years. This method has gained increasing popularity and importance in recent years because the nanofibers obtained by this method are of great importance for tissue engineering.

The process of electrospinning consists of applying a strong electric field at voltages of 5 to 80 kV to a drop of a polymer solution. The polymer solution is placed in a syringe and a polymer droplet forms at the tip of the syringe, i.e. on the capillary nozzle. The electric field is applied between the capillary nozzle and a counter electrode. The electric field thus ensures a change in shape of the droplet, which emerges from the capillary nozzle. A specific form must be achieved, also called Taylor cone. This is only possible if the electric field has overcome the surface tension and the viscosity of the solution. If this has already happened, a continuous jet (polymer jet) forms out of the capillary nozzle. (4).


Fig. 1 Schematic representation of an electrospinning assembly. Ultra-thin spun fibers have diameters of a few nanometers.

These ultra-thin spin fibers have diameters of a few nanometers. The three-dimensional materials produced in this way are suitable scaffolds for tissue engineering. The polymeric Electrospun nanofibers have a number of special properties. For the "Tissue Engineering" it could be shown that the structure of a nanofiber nonwoven fabric is particularly suitable for colonization with cells and their growth. By means of ES a variety of polymers, e.g. Polyamides, polylactones, polyesters and copolymers are spin.In our experiment, the block copolymer polydimethylsiloxane-polyacrylic acid (PDMS-b-PAA) (5) was used. The polydimethylsiloxane-polyacrylic acid is a clinically not yet established polymer. In particular, these polymers gave good results and were in part superior to the established polymers. They have very good properties in promoting cell growth and cell proliferation.

The plants extract *Haberlea rhodopensis* (Haberlea) Fig.2 is a medicinal plant that is considered as an endemic for the Balkan region (especially in Bulgaria, Rhodope).The plants extract *Haberlea rhodopensis* was kindly given from prof. Djilianov from ABI-Sofia,Bulgaria(6) Plants lives in dry areas and has the rare ability to survive without water for a long time, so it is also referred to as the "resurrection plant". The plant contains many active substances, i. many antioxidants, phenolic acids (caffeic acid, hydrocaffeic acid, syringic acid), glucosides and secondary metabolites, entz which have anti-inflammatory, cancer-preventing but also regenerative effects.

Fig. 2 Haberlea rhodopensis

**Crystal violet imaging**

The crystal violet test is a spectrophotometric and morphological method based on the stainability of cells with the dye crystal violet. Crystal violet dissociates in solutions and thereby contains a positive charge. The cation can react with many cell components such as carbohydrates, lipids, lipoproteins and nucleic acids and thus color. The staining was carried out according to the following principle: The medium was removed using a piston-stroke pipette; the coverslips were rinsed with PBS and fixed with 1% formaldehyde in PBS (0.25 mg paraformaldehyde in 25 ml PBS) for 15 minutes at room temperature. This was followed by cell staining for ten minutes with a 0.1% aqueous crystal violet solution. After several washes, the crystal violet excess is removed from the cells. Allow the cells to dry for a few minutes before microscopy them.The work with this dye is gradual. The cells are stained, rinsed several times with water, and then the cells are visualized using a phase-contrast microscope (Axiovert A1, Zeiss, Germany).

**Crystal violet assay**

The electrospun polymeric nanofibers of the PDMS-b-PAA are colonized with human epithelial cells in concentration $4.10^4$ after the cells are incubated with the extract of Haberlea rhodopensis at the concentration of 0.01 mg / ml (a non-toxic concentration). After the incubation period, the cells are monitored for their growth at one day, 3 and 7 day intervals using a 0.1%

aqueous crystal violet solution. The concentration of the dye can be measured on a spectrophotometer (Tecan, Germany) and serves as a measure of the number of viable cells. There is a linear relationship between the measured absorption at 570 nm and the number of cells. By determining the number of viable cells, one can make assertions about cell viability and consequently also about the biocompatibility of the polymeric fibers with the extract, the more cells there are, the more attach well to the surface.

**Cell culture**

The experiment was carried out with the keratinocyte cell line HaCaT (human adult low calcium high temperature). Dulbecco's Modified Eagle Medium (DMEM), which has a glucose content of 4500 mg / l, was used for cell culture. Under sterile conditions, 5 ml of gentamicin (5 mg) -L-glutamine (200 mM) solution and 56 ml fetal calf serum were added to the medium. The complete medium resulted in working concentrations of 2 mM L-glutamine, 10 μg / ml gentamicin and 10% FCS. The cultivation is carried out by incubating the HaCaT keratinocytes in a cell density of approx. $1x10^6$ cells / ml in the complete medium at 37 ° C. and 5% CO2 fumigation in an incubator. After 24 hours, there is a medium change, in which the old medium is sucked off and replaced with a new medium. The cell culture was passaged twice a week with trypsin solution

**Results**

The results of the experiments show that the extract of *Haberlea rhodopensis* has positive effects on the biocompatibility of the polymeric nanofibers, i.e. the cells attach well to the surface within time. Since the plant is considered an endemic, the plants used for the experiment were cultured in vitro. The source of extraction with methanol used here is primarily the leaf material. The crushed leaf material is mixed with 10 g of methanol and extracted in a water bath at 50°C. The process can be accelerated with stirring with a glass rod. The resulting extract is then stored in a refrigerator at -20°C (7).

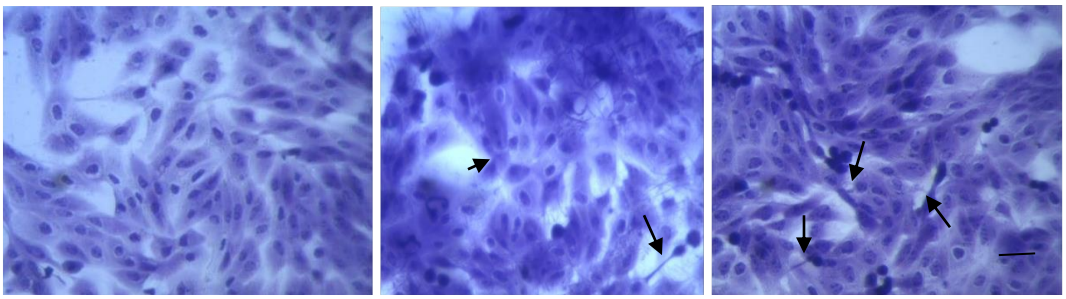
The plant extracts *Haberlea rhodopensis* (Haberlea) Fig.2 is a medicinal plant that is considered as an endemic for the Balkan region (especially in Bulgaria, Rhodope). It lives in dry areas and has the rare ability to survive without water for a long time, so it is also referred to as the "resurrection plant". The plant contains many active substances, i. many antioxidants, phenolic acids (caffeic acid, hydrocaffeic acid, syringic acid), glucosides and secondary metabolites, which have anti-inflammatory, cancer-preventing (8) but also regenerative effects. The results of the experiments show that the extract of *Haberlea rhodopensis* has positive effects on the biocompatibility of the polymeric nanofibers, i.e. the cells attach well to the surface with in time.

## Results and discussion

### Morphological observation

The Microscopic Image For further experiments, cultured cells were suspended and then further diluted. The adherently growing HaCaT cells were spread on coverslips in a 9-well plate. A seeding density of approx. 1 to $4x10^5$ cells / ml must be achieved in the 6-well plate (Costar). Only the 6 wells inside the plate are loaded with cells treated with the extract of Haberlea (test substance). The outer rows of cells (6 wells) are for control. The second set of test samples (3 wells) contains cells seeded on the nanofibers of polydimethylsiloxanes. The new medium for these cells is the extract of *Haberlea rhodopensis*. The third row contains cells that were cultivated directly on the surface of the plate (without PDMS) and with the extract.

The cells were visualized using a phase-contrast microscope (Axiovert A1, Zeiss, Germany). For this cells were first stained with crystal violet, so we can study the structures of the cell are recognizable



A B C

Fig.3 Epithelial cells after discoloration with the crystal violet solution. A: Cells, B: PDMS-b-PAA+*Haberlea rhodopensis and cells,* C: Cells+*Haberlea rhodopensis.*Magnefication 400x

On fig 3A (control,only cells) we faund free space between the cells, the number of cells is not large. The formation of few lamellipodia can be observed, but the cells do not approach each other. Their shape is normal-polygonal epithelial cell shape, they are not broad. On fig 3B we clearly see the nanofibers to which the cells have attached themselves. The cells develop well, can see the lamellipodia (black arrow) of the cells, which is an indication of the interaction of the cells with their neighboring cells. The interactions between the cells are important for their survival, because this creates cell contacts and this leads to tissue formation. The treatment of the cells with the extract has not changed the morphology of the cell culture. The cells have controlled their polygonal morphology, they are large enough, and their cell nuclei are clearly recognizable. There are a few dead cells (darker spots) to observe. The space between the cells is

small, i.e. the number of cells has increased as a result of the good conditions created by the combination of the polymeric fibers and the extract of Haberlea rhodopensis for the cells(9).

On fig 3C the number of cells is a little lower than in sample 3B (PDMS-b-PAA). They are tightly packed together, which is an indication of cell communication. The morphology of the cell culture is good; the cells are small, because of their growth. This results can simple explain. On fig.3C we have just positive action of Haberlea extract compeer to fig 3B where we can see combine effect of PDMS scaffold and Haberlea extract with promote cell viability and biocompatibility.

**Results crystal violet assay**

The crystal violet test is a spectrophotometric method based on the stainability of cells with the dye crystal violet. Crystal violet dissociates in solutions and thereby contains a positive charge. The cation can react with many cell components such as carbohydrates, lipids, lipoproteins and nucleic acids and thus color. The concentration of the dye can be measured on a spectrophotometer (Tecan, Germany) and serves as a measure of the number of viable cells. There is a linear relationship between the measured absorption at 570 nm and the number of cells. By determining the number of viable cells, one can make assertions about cell viability and consequently also about the biocompatibility of the polymeric fibers with the extract, i. the more cells there are, the more attach to the surface. The crystal violet test is carried out similarly, except that it serves to determine the number of viable cells. In order for the results of the experiment to be further used as proof of the biocompatibility of the polymeric fibers, one must measure the number of cells at a time interval. In this experiment, the cells are examined on the first, third and seventh day with a spectrophotometer (Tecan, Germany).

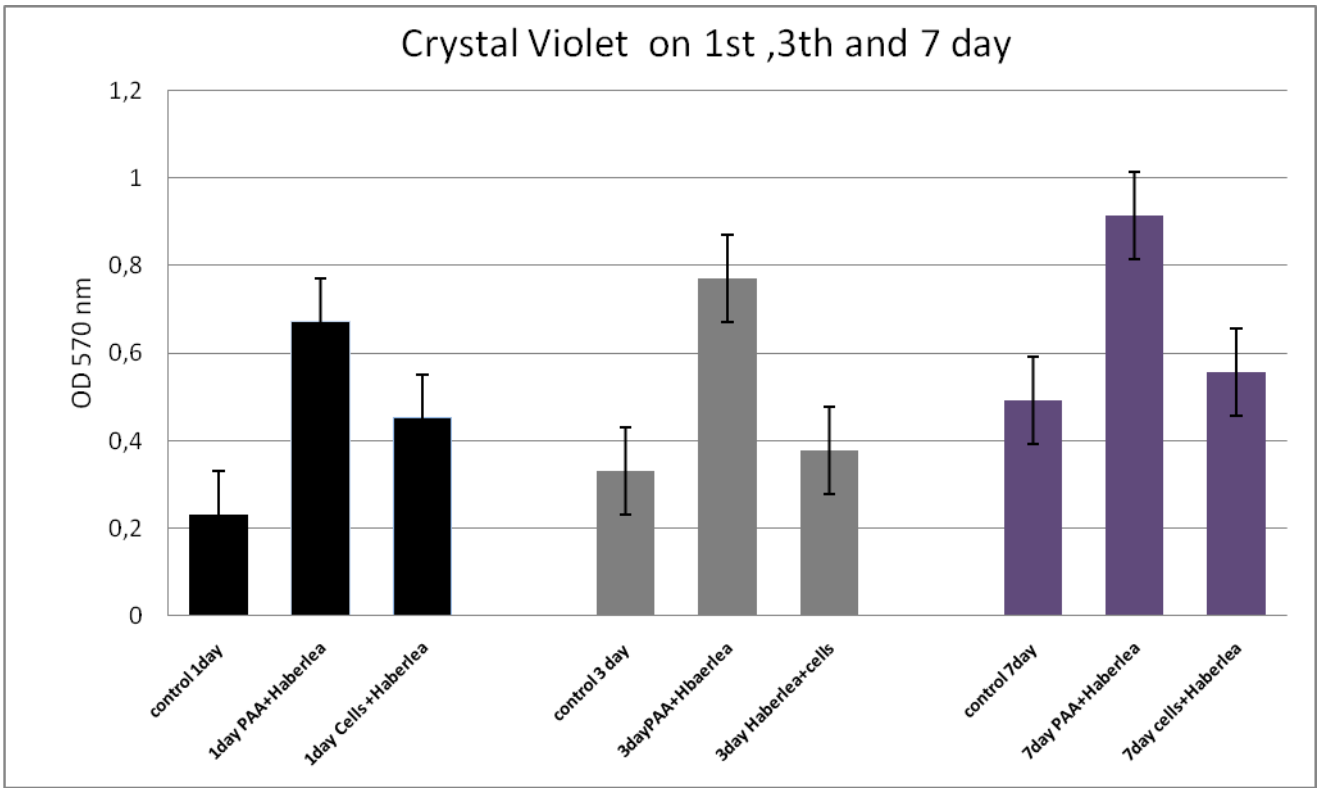



**Fig. 7** Crystal violet assay

From the graph we can see that the number of viable numbers is always the largest at the second column for each experimental day, i. so in the case with the polymeric fibers and the extract of *Haberlea rhodopensis*. From this we can conclude that cell proliferation works best with the combination of the two conditions, PDMS-b-PAA and the extract of *Haberlea rhodopensis*, and this proves to be the best medium for the cells viability and biocompatibility. Results from crystal violet clearly show that we can modulate biocompatibility of PDMS-b-PAA scaffold (10) with extract of *Haberlea rhodopensis* biocompatibility of scaffold depended from hidrophilicity and topography of substratum, also cell viability increase when in medium we have plant extracts because of their antioxidant action of second metabolites like cafeik acid and miconoside (6) with is general poliphenol acid and glycosides in Haberlea extract. Combine effect of good wet ability and good topography plus antioxidant and stimulation effect of Haberlea extract give us one new opportunity for optimize polymer scaffold and use them as medical devise increasing their biocompatibility with cells and tissue.

## Conclusion

Scaffolds are specific surfaces used in tissue engineering. They have to have certain characteristics so that they can fulfill their function. These properties, we can be improved with the addition of herbal extracts. The conjectures we have concretized and focused on a specific

type of plant namely *Haberlea rhodopensis*. As a result, experiments were carried out to determine the viability of the cells under different conditions. The results of the experiments show that the cells are able to attach better to the polymeric fibers using the *Haberlea rhodopensis* extract and to develop further. From this we can conclude that the polymeric fibers treated with an extract of *Haberlea rhodopensis* can be used as a substitute for the previously known surfaces on scaffolds.

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