Multiple effects of electroporation on the adhesive behaviour of breast cancer cells and fibroblasts

Viktoria N. Pehlivanova, Iana H. Tsoneva and Rumiana D. Tzoneva

Abstract

Background: Recently electroporation using biphasic pulses was successfully applied in clinical developments for treating tumours in humans and animals. We evaluated the effects of electrical treatment on cell adhesion behaviour of breast cancer cells and fibroblasts. By applying bipolar electrical pulses we studied short- and long-lived effects on cell adhesion and survival, actin cytoskeleton and cell adhesion contacts in adherent cancer cells and fibroblasts.

Methods: Two cancer cell lines (MDA-MB-231 and MCF-7) and one fibroblast cell line 3T3 were used. Cells were exposed to high field intensity (200 - 1000 V/cm). Cell adhesion and survival after electrical exposure were studied by crystal violet assay and MTS assay. Cytoskeleton rearrangement and cell adhesion contacts were visualized by actin staining and fluorescent microscope.

Results: The degree of electroporation of the adherent cells increased steadily with the increasing of the field intensity. Adhesion behaviour of fibroblasts and MCF-7 was not significantly affected by electroporation. Interestingly, treating the loosely adherent cancer cell line MDA-MB-231 with 200 V/cm and 500 V/cm resulted in increased cell adhesion. Cell replication of both studied cancer cell lines was disturbed after electroporation. Electroporation influenced the actin cytoskeleton in cancer cells and fibroblasts in different ways. Since it disturbed temporarily the actin cytoskeleton in 3T3 cells, in cancer cells treated with lower and middle field intensity actin cytoskeleton was well presented in stress fibers, filopodia and lamellipodia. The electroporation for cancer cells provoked preferentially cell-cell adhesion contacts for MCF-7 and cell-ECM contacts for MDA-MB-231.

Conclusions: Cell adhesion and survival as well as the type of cell adhesion (cell-ECM or cell-cell adhesion) induced by the electroporation process is cell specific. The application of suitable electric pulses can provoke changes in the cytoskeleton organization and cell adhesiveness, which could contribute to the restriction of tumour invasion and thus leads to the amplification of anti-tumour effect of electroporation-based tumour therapy.

Keywords: Breast cancer cells, Fibroblasts, Actin cytoskeleton, Electroporation
New Polyvinyl alcohol-based hybrid materials for biomedical application

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ABSTRACT

Polyvinyl alcohol (PVA), a hydrophilic, biodegradable and biocompatible synthetic polymer, has been widely used in different areas of the biotechnological and biomedical field. PVA is very sensitive towards moisture and as a result the film strength is reduced, which is strongly undesirable for biomedical applications. To overcome this problem, new hybrid materials based on PVA and γ-aminopropyltriethoxysilane (APTEOS), 1-mercaptopropyltriethoxysilane (MPTEOS) and tetraethoxysilane (TEOS) were prepared. Physicochemical surface characterization of the materials was done by measuring the water contact angle of the materials. The new materials were tested for potential use as matrices in tissue engineering applications. Compatibility and the cell adhesive behavior (actin cytoskeleton) of 3T3 cells as a function of surface functional groups were studied.

The synthesized PVA/APTEOS materials proved efficient for use in tissue engineering applications. © 2012 Elsevier B.V. All rights reserved.

1. Introduction

Polyvinyl alcohol (PVA), a hydrophilic, biodegradable and biocompatible synthetic polymer, has been widely used in different areas of the biotechnological and biomedical field due to its excellent chemical and physical properties, easy processing technique and low cytotoxicity. However, PVA is very sensitive towards moisture and as a result the film strength is reduced, which is highly undesirable for biomedical applications [1]. The incorporation of networked silica using different orthosilicic acid, TEOS, APTEOS and MPTEOS as precursors via the sol-gel method into PVA matrix is a way to improve significantly the properties of the used matrices [1-5]. Using the sol-gel method, it is possible to hybridize the organic and inorganic components homogeneously which has an impact on the physicochemical and mechanical properties of the hybrid materials [6].

Recently hydrogels as PVA have become attractive to the new field of tissue engineering and regenerative medicine as matrices for repairing and regenerating a wide variety of tissues and organs [7]. In tissue engineering, PVA-based scaffolds have been studied to substitute the current available artificial grafts. The appearance and feel of PVA hydrogel are similar to those of native arterial tissue and this makes it very adequate for vascular implanting [8,9]. Due to its tissue-like elasticity and mechanical strength PVA has been proposed as a promising biomaterial suitable for tissue mimicking [10]. Surface modification of hydrogels with different functional groups can lead to improvement of some physico-chemical properties like time of gelation and strength of hydrogels (thiol content) [11] or can alter cell adhesion (SH-, OH-, NH- groups) [12,13]. Overall morphology of adherent cells is a key characteristic to test a given polymer material for its application in biotechnology and tissue engineering. Staining the cytoskeleton organization is most common way to understand the adhesion behavior on polymer surface.

The aim of present work is to investigate the adhesive behavior of 3T3 cell line onto newly developed PVA-based hybrid materials in dependence on their functionality and surface physico-chemical properties for application in tissue engineering.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA) (Sigma- Aldrich; 87-88% hydrolyzed, Mw=13,000–23,000 mol−1), HNO3 (Riedel de Haën, Standard solution 2 mol/L), Tetraethyl orthosilicate (Fluka), γ-aminopropyltriethoxysilane and 3-mercaptopropyltriethoxysilane were used as received without further purification.

2.2. Synthesis of PVA based hybrid materials

2.2.1. Synthesis of PVA/TEOS and PVA/MPTEOS hybrid matrix

PVA (5 g) was dissolved in 95 mL deionized water while heating for 20 min at 80 °C. The silica sol was produced by hydrolyzing partially TEOS and MPTEOS (0.93 mL) in acidified...
Elastic multiblock copolymers for vascular regeneration: Protein adsorption and hemocompatibility

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Abstract. Hemocompatibility of elastic multiblock copolymers PDC, based on poly(p-dioxanone) (PPDO)/poly(ε-caprolactone)-segments, capable of a shape-memory effect, and PDD, based on PPDO/poly(adipinate-alt-1,4-butanediol)-co-(adipinate-alt-ethylene glycol)-co-adipinate-alt-diethylene glycol)-segments, was studied in order to assess their suitability for an application aiming at blood vessels regeneration. The results were compared with polypropylene (PP) which is a widely used blood-contacting material for devices as blood oxygenators and dialysis tubes. Protein adsorption studies showed diverse blood plasma proteins in a relatively high amount on both elastic polymers compared to the poor amount of plasma proteins adsorbed on PP. Study of the coagulation system revealed high thrombin formation on PDC and no difference in plasma kallikrein activation between elastic multiblock copolymers and the reference PP. Activation of complement system was higher for PDC followed by PDD and lower for PP. However, platelet adhesion and activation were hardly suppressed on the multiblock copolymers compared to the PP surface, where the number of adhered platelets and the activation rate were significant. The present results reveal that the tested multiblock copolymers with improved elastic properties and shape-memory capability (PDC) show low thrombogenicity and are promising candidates for vascular tissue engineering.

Keywords: Hemocompatibility, degradable elastic polymer, protein adsorption, coagulation, thrombin formation, platelet adhesion/activation, shape-memory polymer

1. Introduction

Cardiovascular diseases remain the leading cause of mortality in western nations. Despite significant improvements and the broad adoption of minimally invasive treatments such as balloon angioplasty and stenting, the therapeutic gold standard for patients with far advanced heart diseases is the coronary

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A novel pH sensitive water soluble fluorescent nanomicellar sensor for potential biomedical applications

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ABSTRACT

Herein we report on the synthesis and sensor activity of a novel pH sensitive probe designed as highly water-soluble fluorescent micelles by grafting of 1,8-naphthalimide–rhodamine bichromophoric FRET system (RNI) to the PMMA block of a well-defined amphiphilic diblock copolymer–poly(methyl methacrylate–co–poly(methacrylic acid) (PMMA\textsubscript{85}–b–PMAA\textsubscript{25}). The RNI-PMMA\textsubscript{85}–b–PMAA\textsubscript{25} adduct is capable of self-assembling into micelles with a hydrophobic PMMA core, containing the anchored fluorescent probe, and a hydrophilic shell composed of PMAA block. Novel fluorescent micelles are able to serve as a highly sensitive pH probe in water and to internalize successfully HeLa and HEK cells. Furthermore, they showed cell specificity and significantly higher photostability than that of a pure organic dye label such as BODIPY. The valuable properties of the newly prepared fluorescent micelles indicate the high potential of the probe for future biological and biomedical applications.

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1. Introduction

Determination of pH is one of the most important analytical methods in the chemical laboratories and in the industry. The pH is a key parameter in clinical analysis, food production, biotechnological processes, waste water treatment procedures, environmental and life sciences. Intracellular pH plays a critical role in many cellular events, including cell growth and apoptosis, ion transport and homeostasis and enzymatic activity. Abnormal pH values are associated with inappropriate cell function, growth, and division and are observed in some common disease types such as cancer and Alzheimer’s. Hence the determination of pH has attracted increasing interests.

Although the potentiometric pH sensor is well-established for routine pH measurements, it possesses some limitations as regarding miniaturized and disposable devices, work in a strong electromagnetic field, high throughput screening, presence of organic matter or selectivity in high pH media. Indeed, in some applications, pH electrode is irreplaceable, but in a number of researches and technological tasks fluorescence probes could be an alternative to overcome the above mentioned limitations. Currently a very intensive research on pH fluorescent chemosensors is focusing on the synthesis of water-soluble fluorophores, that can be miniaturized, are disposable or calibration-free sensors, and are widening the dynamic pH range either by design of new dyes or by combining different sensors in one fluorescence probe.

A large number of organic dyes have been developed for bioimaging purposes to investigate the main processes at the tissue, cellular and molecular level. Based on their chemical structure, they can be divided into several classes such as cyanine, porphyrin, squaraine, BODIPY, and xanthenes. The main disadvantages of commonly used fluorescent organic dyes are their non-specific to target tissue, instability and toxicity which limit further biomedical application because of potential carcinogenesis, low threshold of photobleaching, and lack of functional groups for further...

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Cell adhesive behavior of PVA-based hybrid materials with silver nanoparticles

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Abstract

Hybrid materials based on polyvinyl alcohol (PVA) and tetraethoxysilane (TEOS) with embedded silver nanoparticles (AgNps) were prepared using sol-gel method. The hybrid materials with three different silver concentrations from 0.36 mg/mL to 1.8 mg/mL were characterized by TEM, SEM and TGA analyses. Physico-chemical surface characterization of the materials was done by measuring the water contact angle of the materials and ATR. The materials were tested as matrices for potential use in tissue engineering field. The cytotoxicity of the materials and the cell adhesion (actin cytoskeleton) of J73 cells as a function of silver nanoparticles content were studied. The synthesized PVA/TEOS/AgNps materials were proven to be efficient for use in tissue engineering applications since they exhibit low cytotoxicity and sufficient cell adhesion.

1. Introduction

Silver has been used since many years due to its strong antimicrobial properties. With the development of nanotechnology, the interest towards silver nanoparticles (AgNps) as a biocidal agent is increasing due to its higher antimicrobial properties towards Gram-positive and Gram-negative bacteria [1–4] and low toxicity to humans compared to other heavy metal ions such as chromium, manganese, cadmium etc. [5,6]. Many authors have demonstrated the efficacy of using silver nanoparticles for biomedical applications for instance for wound healing [7,8] where silver nanoparticles promote wound healing through their powerful antibacterial properties as well as their ability to decrease inflammation [7,9]. However, there are arising evidences that silver nanoparticles exhibit toxic effect to eukaryotic cells [10,11]. Therefore, it is important the preparation of appropriate for biomedical application material possessing good antibacterial activity and low toxicity to eukaryotic cells. Polymer nanocomposites are advanced functional materials composed of nanoparticles dispersed inside the polymeric matrix [12,13], resulting in a hybrid material which combines the suitable properties of both compounds [14]. They could be good materials of choice to solve the above problems with the cytotoxicity of Ag nanoparticles. Among the numerous nanoparticles that have been used as polymer functionalizing agent, silver nanoparticles represent the most sought-after (nano)material. Nevertheless, numerous practical applications of silver nanoparticles require their entrapment on various substrates and matrices. From this point of view, polymers are materials of first choice because of their specific morphology, chemical and structural nature with long polymeric chains allowing incorporation and fine dispersion of nanoparticles. Additionally, the suitable functional groups of polymers can be used as targeted reactive sites for the controlled one-step synthesis of nanoparticles [15]. In order to fully exploit the properties of silver nanocomposites, they should be well dispersed within the polymer matrix without the formation of large aggregates, which otherwise dramatically could reduce the antimicrobial effect of silver. For the same reason, the size of the nanoparticles should be as small as possible with a narrow size distribution [16]. The Ag nanoparticles are incorporated and stabilized in the polymer matrix and is not supposed to be released. The advantages of those nanocomposite materials are that the microorganisms can be killed upon direct contact without releasing Ag nanoparticles that might be toxic not only to bacteria but also to mammalian cells [17,18].

Polyvinyl alcohol (PVA) is a good host material for metal, due to its excellent thermostability, chemical resistance and hydrophilic properties. This biodegradable and biocompatible synthetic polymer has been widely used in different areas of the biomedical field due to its excellent chemical and physical properties, easy processing technique and low cytotoxicity [19]. In our previous investigation [1], we studied the antibacterial properties of PVA based AgNp hybrid materials against etalon strains of three different groups of bacteria—Staphylococcus aureus (Gram-positive bacteria), Escherichia coli (Gram-negative bacteria) and Pseudomonas aeruginosa (non-ferment Gram-negative bacteria), which are commonly found in hospital environment. The hybrid materials showed a strong bactericidal effect against the above bacterial strains.

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The role of alternating current electric field for cell adhesion on 2D and 3D biomimetic scaffolds based on polymer materials and adhesive proteins

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Tissue engineering principles suggest the formation of 3D scaffolds based on polymer fibers and adhesive proteins. These scaffolds aim to mimic the native extracellular matrix and thus providing a favorable environment for cell attachment and proliferation. The application of an electric field (EF) can influence the quantity and the spatial orientation/conformation of adsorbed proteins, which could lead to changes in their functions. We study the influence of alternating current (AC) EF on the adsorption of fibronectin onto poly(etherimide) (PEI) electrospun fiber materials in 3D structures and subsequent cell adhesion. The results are compared with 2D PEI material and glass surface. 3D scaffolds adsorbed a lower amount of fibronectin than 2D film or glass. Application of AC EF with a frequency of 1 Hz decreased the adsorption of fibronectin. Cell adhesion on 3D materials was reduced compared with 2D film and glass. The application of EF with frequencies between 1 and 10 Hz improved cell adhesion on both 2D and 3D materials.

I. INTRODUCTION

Cell adhesion to biomaterials is a desirable process in many tissue engineering applications to regenerate non-functional or damaged tissues or organs.1-2 The application of tissue engineering principles in regenerative medicine enables the formation of 3D scaffolds based on polymer fiber materials similar to the structure of native extracellular matrix (ECM). Since the scaffold acts as an artificial ECM, it should mimic certain functions of natural ECM to facilitate cell adhesion, proliferation, differentiation, and formation of functional tissue.3 A high surface-to-volume ratio and porosity of the fibrous scaffold are thought to enhance adhesion and colonization of cells within the matrix and support an efficient exchange of nutrients and products of metabolism within the matrix and with the environment.4,5 Material design of scaffolds is one of the key technologies for tissue engineering in terms of chemical composition and physical structure. Poly(etherimide) (PEI) has become a good candidate for wide range of biomaterial applications especially for extra corporeal systems such as oxygenators.6-11 The investigations on its biocompatibility have shown that polyimides do not exert any significant level of cytotoxicity or hemolysis and allow the attachment and growth of cells.12-14 PEI also possesses considerable mechanical strength and thermal stability,15 which makes PEI suitable for steam sterilization. Furthermore, PEI has very good membrane-forming properties10 and can be chemically functionalized to modify surface properties or to link bioactive molecules. In this way, the resulting polymer material easily can be adapted to a specific application such as contact with blood or tissue cells.15-17

Electrospinning is a well-established process, by which ultrafine fibers at the very upper limit of the fiber diameter range of natural ECM (upper nanometer to lower micrometer range) are produced using electrically charged droplets of melt polymer or their solution.18 One promising approach to mimic the structure of native ECM and to support...
EFFECT OF ERUFOSINE ON THE REORGANIZATION OF CYTOSKELETON AND CELL DEATH IN ADHERENT TUMOR AND NON-TUMORIGENIC CELLS

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ABSTRACT
Cell adhesion plays a key role in tumor progression and its control could diminish the tumor metastases. The action of erufosine on cell survival, reorganization of cytoskeleton, and apoptosis was analyzed in breast cancer and mammary epithelial cells. Breast cancer cell lines MDA-MB-231 and MCF-7 as well as the non-tumorigenic line MCF-10A were treated with erufosine (5 µmol/L to 15 µmol/L). MTS test, FACS analysis, actin, tubulin and DAPI staining were performed. For MDA-MB-231 erufosine provoked apoptosis and actin reorganization, while MCF-7 and MCF-10A were less sensitive to the action of erufosine. The organization of microtubules was not affected by erufosine treatment. The cytotoxic action of erufosine on the investigated cells was cell-specific. The highly invasive MDA-MB-231 cell line was found to be most sensitive, since 15 µmol/L erufosine caused actin cytoskeleton reorganization and apoptosis.


Keywords: erufosine, breast cancer cells, cytoskeleton, apoptosis

Introduction
The alkylphosphocholines (APC) are a new group of antitumor agents, which show cytotoxic activity against different tumor cell lines in vitro and antineoplastic activity in vivo (1, 12). Unlike standard chemotherapeutics and irradiation, which work on the DNA level, APC are membrane-operating agents which inhibit protein kinase C and modulate the signal transduction pathways originating from the membrane (5). They induce apoptosis in many tumor models (7, 11) and selectively damage leukemic cells without affecting (by the same concentration) the normal cells of the bone marrow (10). Recently, a new alkylphosphocholine analog was obtained, called erufosine-N,N,N-trimethylpropylammonium (ErPC₃, erufosine). This is the first injected agent which loses its hemolytic properties and shows increased therapeutic ratio in vivo (2). The drug can even stimulate the production of hematopoietic progenitor cells (6, 15). Cell adhesion is a fundamental process, which, in non-tumorigenic cells, plays a crucial role in the cell growth and survival because it sustains the organization of tissues and organs. For tumorigenic cells the presence of adhesion contacts is not a requirement for cell growth and survival (4). The changes in the cells’ adhesion behavior determine their modified morphology and migration behaviors which affect the cells’ invasive properties during all the stages of tumorigenesis. Thus, the manipulation of cell adhesiveness is an important prerequisite for the control of cancer cell growth and invasion. The cytoskeleton plays a crucial role for the manifestation of cell adhesiveness. Actin filaments, filopodia and lamelipodia ensure the cell adhesion, spreading...
Novel nanosized water soluble fluorescent micelles with embedded perylene diimide fluorophores for potential biomedical applications: Cell permeability, localization and cytotoxicity

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ABSTRACT

Novel biocompatible water-soluble fluorescent micelles with embedded perylene diimides (PDI) for intracellular applications have been prepared by self-assembly of amphiphilic poly(vinyl alcohol)-b-poly(acrylonitrile) (PVA-b-PAN) copolymers in the presence of synthesized fluorophores. Amphiphilic PVA-b-PAN copolymers were obtained by selective hydrolysis of well-defined poly(vinyl acetate)-b-poly(acrylonitrile) (PVAc-b-PAN) copolymer. The preparation of the novel fluorescence micelles consisting of PVA hydrophilic shell and PAN hydrophobic core with incorporated PDI fluorophores has been confirmed by DIS and TBM analysis. The cytotoxicity of the water-soluble fluorophores and their internalization into living cells depending on the micellar concentration have been tested. It was shown that they could successfully enter in living cells without destroying their morphology. The results obtained indicate that the novel water-soluble fluorescent micelles with embedded PDI fluorophores would be suitable for potential intracellular biomedical applications.

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1. Introduction

Perylene-3,4,9,10-tetracarboxylic diimides (PDI) have attracted an increasing interest in the past decades, and this can be traced back to their appealing properties, e.g., high electron affinity, large electron mobility, excellent thermal and oxidative stability, high molar absorptivity and quantum yield of fluorescence [1]. They are easy of synthetic modification [2] and widely used n-type materials for organic electronic devices such as solar cells [3–5], field-effect transistors [6–8], light-emitting diodes [9–11]. They have been also used in liquid crystals [12,13], as chemosensing materials [14–16] and dyes in photodynamic therapy [17]. Due to their unique properties PDI chromophores could be successfully applied in biological environment as high-performance fluorescent labels. The serious drawback of most of the synthetic dyes for their processing and material science applications is their low solubility in the common solvents and aqueous media [18,19]. Usually the solubility can be improved with the long-tail or swallow-tail configuration that is obtained by long alkyl substitutions in N-position of the dye [20–22]. For a biological application, the water solubility of PDI chromophores is essential. Different water-soluble PDI chromophores possessing hydrophilizing substituent as a sulfonic acid part [23], quaternized amine groups [24], polyethylene glycol attached to the chromophore [25] have been reported. In many cases, no fluorescence in water for these perylene chromophores has been detected. The inserting of polar side chains on the perylene bay-area is another way to enhance the water solubility of the PDIs and to reduce the amount of their self-aggregation [26,27] followed by encapsulation within dendritic shell thus preventing the possible intermolecular interaction [26]. Therefore, the choice of an appropriate synthetic strategy where the fluorescence properties of water soluble PDI chromophores will be retained and the self-aggregation will be reduced is of crucial importance. However, the synthetic approaches for preparation of such water soluble chromophores are often hard and multistep time consuming processes.
Modification of *Rapana thomasiana* hemocyanin with choline amino acid salts significantly enhances its antiproliferative activity against MCF-7 human breast cancer cells†

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This is the first study on the interactions of ionic liquids with large metalloproteins, in particular hemocyanins (Hcs). At first, complexes of a Hc from *Rapana thomasiana* (RTh) with a series of biocompatible choline amino acid salts [Choll][AA] were obtained. Applying UV-vis spectroscopy, Fourier-transformed infrared spectroscopy and differential scanning calorimetry the effect of these organic salts on the structure and thermal stability of RTh was assessed. Then, the cytotoxic effect of RTh-[Choll][AA] on breast cancer cells (MCF-7) and 3T3 fibroblast cells (non-cancerous) was evaluated. We found that all [Choll][AA] induced clear time- and concentration-dependent alterations in the RTh conformation. The conformation and the thermal stability of IL-modified RTh depend strongly on the type of the anion of the tested compounds. All [Choll][AA]-modified RThs exhibited lower thermal stability than the native RTh. At the same time, we established a good correlation between the structure of RTh and its antitumor activity. Namely, RTh-[Choll][AA] complexes exhibited enhanced antiproliferative activity toward the MCF-7 cell line. The observed antiproliferative effect was cell specific and the compounds have no effect or in some cases have stimulatory effect on fibroblasts.

1. Introduction

Hemocyanins (Hcs) are respiratory type-3 copper proteins found in the hemolymph of molluscs and arthropods. Molecules of molluscan Hcs are structured as decamers (cephalopods) or didecamers (gastropods) of subunits, all comprising of eight functional units (FLs). Each FU contains a binuclear copper active site, complexed with six histidine residues, and capable to bind dioxygen reversibly. Hcs are known for their remarkable immunostimulating and immunomodulating properties and some of them are successfully used in oncotherapy and have potential as vaccine adjuvants. The functionality of these respiratory proteins can be altered by modification of their structure and conformation. For example, it has been found that the modification of the Hc form limpet *Fissurella latimarginata* with sodium periodate enhanced its immunogenicity, and Hc from scorpion *Pandinus imperator* acquired a phenoloxidase activity after treatment with sodium dodecyl sulfate. In addition, it has been reported that treatment of a Hc from *Rapana thomasiana* (RTh) with detergents, resulted in a limited proteolysis or lyophilization induced change in the protein conformation and enhanced enzyme activity.

As can be seen, depending on the environment and the conditions, Hcs due to their complex structure can be involved in various chemical and biochemical reactions (e.g. oxygen transportation, induction of immune response, development of biosensors, wound healing processes, etc.). Not only the primary structure and carbohydrate composition but also the correct folding of the whole protein is indispensable factor responsible for its activity. It is noteworthy to be mentioned that, except the effect of SDS, the effect of other organic salts on the secondary structure and the biological activity of Hcs, particularly cell proliferation, have not been estimated yet.

Potentially good effectors for hemocyanin modification could be ionic liquids (ILs). They are molten salts comprising of an organic cation and an organic or an inorganic anion and characterize with melting points below 100 °C, low vapour
Antitumor Lipids—Structure, Functions, and Medical Applications

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Abstract

Cell proliferation and metastasis are considered hallmarks of tumor progression. Therefore, efforts have been made to develop novel anticancer drugs that inhibit both the proliferation and the motility of tumor cells. Synthetic antitumor lipids (ATLs), which are chemically divided into two main classes, comprise (i) alky/phaspholipids (APLs) and (ii) alkyphosphocholines (APCs). They represent a new entity of drugs with distinct antiproliferative properties in tumor cells. These compounds do not interfere with the
Angiogenic potential of endothelial and tumor cells seeded on gelatin–based hydrogels in response to electrical stimulations


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Abstract. Angiogenesis is one of the key processes during development, wound healing and tumor formation. Prerequisite for its existence is the presence of endogenous electrical fields (EFs) generated by active ion transport across polarized epithelia and endothelia, and appearance of the transcellular potentials. During angiogenesis cellular factor as endothelial growth factor (VEGF), synthesis of adhesive proteins and membrane metalloproteinases (MMPs) govern the angiogenic response to different external stimuli as biomaterials interactions and/or exogenous EF. Gelatin-based hydrogels with elasticities comparable to human tissues have shown to influence cell behavior as well as cell attachment, protein synthesis, VEGF and MMPs production after the application of EF. Gelatin-based matrices with 3 (G10.LNO3), 5 (G10.LNCO3), and 8 (G10.LNCO8) fold excess of isocyanate groups per mol of amine groups present in gelatin were used. Human umbilical endothelial cells (HUVEC) (Lonza Basel, Switzerland) and highly invasive breast cancer MDA-MB-231 cells (ATCC® HTB-26™) were used. For an estimation of the amount of VEGF released from cells a commercially available VEGF ELISA (Thermo Fischer Scientific, Germany) kit was used. Fibronectin (FN) enzyme immunoassay (EIA) was used to analyze the secreted amount of FN by cells seeded on the materials. Secreted MMPs were analyzed by zymography. Gelatin-based hydrogels attracted HUVEC adhesion and diminished the adhesion of MDA-MB-231 cells. The applied direct current (DC) EF induced an almost 5-fold increase in VEGF production by HUVEC seeded on gelatin-based hydrogels, while in contrast, the applied EF decreased the production of VEGF by cancer cells. FN synthesis was elevated in HUVEC cells seeded on gelatin-based materials in comparison to FN synthesis by cancer cells. HUVEC seeded on gelatin hydrogels showed an expression mainly of MMP-2. The application of EF increased the production of MMP-2 in HUVEC seeded on gelatin materials. In contrast, for MDA-MB-231 the production of MMPs on gelatin materials was lower compared to control materials. With the application of EF the levels of MMP-9 decreased but MMP-2 expression raised significantly for gelatin materials. Overall, the results showed that studied gelatin materials suppressed attachment of cancerous cells, as well as suppressed their angiogenic potential revealed by decreased VEGF and MMP production. Thus, this study approved gelatin-based hydrogels with proper elasticity characteristics and different degradation behavior as useful matrices for use in vascular tissue regeneration or in restriction of tumor growth after tumor resection.

Keywords: Angiogenesis, gelatin hydrogels, VEGF production, FN synthesis, MMPs expression

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Novel electrically conducting 2-hydroxyethylcellulose/polyaniline nanocomposite cryogels: Synthesis and application in tissue engineering

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**ABSTRACT**

Novel electrically conducting 2-hydroxyethylcellulose/polyaniline (HEC/PANI) nanocomposite cryogels were fabricated via the combination of cryogenic treatment and photochemical crosslinking. PANI nanofibers (one-dimensional tubes and three-dimensional particles) were synthesized via oxidative polymerization of aniline in aqueous media and, then, embedded in the HEC matrix. The effect of PANI content and morphology on the gel fraction yield and electrical conductivity of material was studied. Nanocomposite cryogels of high gel fraction yield (65–95%) and rather high electrical conductivity (0.02–0.1 S/m) were obtained by using a relatively small amount (0.5–3 wt% to HEC) of pre-formed PANI nanofibers. The behavior of 1929 cells adhered on HEC/PANI cryogels in the presence of electric field were also investigated. Cytotoxicity test showed very good survival and proliferation of cells on cryogels, while the electrical stimulation triggered changes in cell morphology as well as a specific alignment of cells in parallel to the electrical field.

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1. Introduction

Presently, the research focused on novel biomaterials is booming worldwide with the general belief that their applications in medical devices will form one of the greatest areas of expansion over the next decade (Schoen & Lemons, 2013). Various natural and synthetic biocompatible polymers have been widely used in biomedical applications, including surgical sutures, bone fixation devices, vascular grafts, artificial skin, drug delivery systems, gene delivery systems, diagnostic applications, etc. (Seal, Otero, & Panitch, 2001; Kumari, Yadav, & Yadav, 2010; Tian, Tang, Zhang, Chen, & Jing, 2012; Kabanov, Batrakova, & Alakhov, 2002). In particular, degradable materials from synthetic polymers such as poly(e-lactic acid) (PLLA), poly(e-caprolactone) (PCL) and poly(ε-glycolic acid) (PLGA), and/or natural biological polymers such as alginate, chitosan, collagen, and fibrin have intensively been exploited as tissue engineering scaffold, providing mechanical support, shape, and cell-scale architecture for neo-tissue construction in vitro or in vivo (Furth, Atala, & Van Dyk, 2007). Biopolymer scaffolds combine several functions including biocompatibility with the host tissue, controlled biodegradability with non-toxic degradation products, adequate porosity for the transportation of small molecules, optimal mechanical strength and controllability during cell growth, implantation and sterilization.

Electrical stimulation (ES) has been employed as a very efficient approach to regulate cellular functions. Endogenous electrical field (EF) plays an integral role in maintaining normal biological functions such as signaling in the nervous system, muscle contraction, and wound healing (McCaig, Rajniecek, Song, & Zhao, 2005; Tzoneva, 2014; Guimard, Gomez, & Schmidt, 2007). Application of exogenous EF in clinical settings has been used as an alternative treatment to promote wound healing of skin and corneal epithelium (Ojingwa & Isseroff, 2003), to reduce chronic pain in arthritis and headaches (Rushon, 2002), to treat diseases such as Parkinson (Loher et al., 2002), and to enhance nerve regeneration (Sisken, Walker, & Orgel, 1993). In addition, some of us have demonstrated that the application of alternative EF improves the adhesion of fibroblasts on 3D poly(etherimide) electropositive fiber materials (Pehlivanova et al., 2013).

In the last two decades, the class of conducting polymers (CP) has been studied for many biomedical applications (Guimard et al., 2007). CP possess electrical and optical properties similar to those of metals and inorganic semiconductors and can be easily synthesized and processed. These unique characteristics make...
Rapana thomaisiana hemocyanin modified with ionic liquids with enhanced anti breast cancer activity

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ABSTRACT

This is the first study on the surface modification of a hemocyanin from marine snail Rapana thomaisiana (RtH) with series of imidazolium-based amino acid ionic liquids [emim][AA]. We monitored the induced by [emim][AA] conformational changes in RtH molecule and evaluated the effect of these ionic liquids (ILs) on the protein thermal stability. The cytotoxicity of all obtained RtH-[emim][AA] complexes was assessed toward breast cancer cells (MCF-7) and murine fibroblasts (3T3).

As a whole, even small amounts of the tested ILs altered the secondary structure of RtH. The thermal denaturation of RtH in presence of [emim][AA] displayed multi-component transitions, which were shifted toward lower temperatures in comparison to those estimated for the native RtH. The profiles of the RtH-IL calorimetric curves show a clear dependence on the structure of the added salts. In addition, all RtH-[emim][AA] complexes exhibited an enhanced anti-proliferative activity toward MCF-7 cells in comparison to that of the native RtH. The best results are observed for RtH-[emim][Leu], RtH-[emim][Trp] or RtH-[emim][Ile], which applied in concentration of 700 μg/mL inhibited the MCF-7 cell viability (for 24 h) by 66, 63 and 53%, respectively. In addition, these IL-RtH complexes were less cytotoxic to 3T3 cells, i.e. they exhibited some cell specificity.

1. Introduction

Ionic liquids (ILs) are molten salts comprising of an organic cation and an organic or an inorganic anion. They are considered as environmentally friendly media due to their low melting points (<100 °C), low vapor pressures, non flammability, thermal and chemical stabilities, controlled miscibility with organic solvents and water, etc. [1,2]. It is noteworthy to be mentioned that, being highly polar yet noncoordinating solvents, ILs are able to dissolve wide range of inorganic and organic materials, which makes them suitable media for synthetic organic and biocatalytic reactions and extraction solvents for biopolymers, secondary plant metabolites and/or synthetic products in separation technologies [3–6]. By altering the structure of either the cation or the anion of ILs, a directed “tuning” of their physicochemical properties can be achieved, which makes ILs applicable in many other fields beside synthesis and catalysis. For example, some ILs are industrially applied as electrolytes for batteries, engineering fluids (hydraulic, cleaning and cooling liquids), liquid supports for storage of gases, etc. [6]. In recent years, an increasing attention has been paid to the biomedical application of ILs. For example, there are reports on the synthesis of pharmaceutically active ILs with improved solubility, bioavailability and pharmacokinetics and increased anti-bacterial, anti-inflammatory and anti-allergic properties in comparison to their conventional counterparts [7,8]. In addition, it has been shown that numerous pure ILs are potent antimicrobial and anti-cancer agents [9,10]. In last decade, with the fast development of biotechnological drugs (mono- and bifunctional antibodies, fusion and plasma derived proteins, growth factors, vaccines, etc.), a special attention has been paid to the methods for their stabilization. Ionic liquids have potential to be a good alternative to conventional protein stabilizers (polys, carbohydrates, surfactants, salts, etc.) [11]. For example, Kumar and Venkatesu have observed that some short-chain alkyl imidazolium-based and
Cytotoxicity and antibiofilm activity of SiO2/cellulose derivative hybrid materials containing silver nanoparticles

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Abstract: Hybrid materials based on tetraethyl orthosilicate (TEOS), hydroxypropyl cellulose (HPC), or hydroxypropyl methylcellulose (HPMC) with silver nanoparticles were synthesized. They were analyzed and characterized using differential thermal analysis and thermogravimetry, atomic force microscopy, and static contact angle measurements. It was experimentally demonstrated that the silver-doped hybrid materials have pronounced antibacterial behavior by studying the reduction of Pseudomonas aeruginosa PA01 biofilm formation on the tested materials. The results revealed biofilm reduction of 35.7% and 30% by SiO2/HPC/2.5% Ag and SiO2/HPMC/2.5% Ag hybrid materials, respectively, compared to the control. Cytotoxicity of examined materials and actin cytoskeleton organization of fibroblasts seeded on the materials was studied as a function of material properties as the type of surface functional groups and silver content. The obtained hybrid materials with low silver content proved efficient in tissue engineering applications since they showed good antibacterial and nontoxic properties for eukaryotic cells.

Key words: Silver nanoparticles, silica hybrid materials, antibacterial activity, cytotoxicity, cell adhesion, tissue engineering

1. Introduction
Bacterial infections of wounds are huge health problems and are due to the adhesion of pathogenic bacteria, which form biofilms. Microorganisms in such biofilms possess competitive advantages such as high resistance to antibiotics. Currently, different polymer dressings impregnated with silver nanoparticles (AgNPs) are being established as promising antibiofilm reducing agents (Campoccia et al., 2013; Velazquez-Velazquez et al., 2015). Silver-containing materials are widely used as antimicrobial agents for different biomedical applications like wound-healing devices (Ip et al., 2006; Tian et al., 2007), catheters (Hachem et al., 2003), dental materials (Hernandez-Sierra et al., 2008), and stents (Multanen et al., 2006) due to their ability to kill a broad spectrum of bacteria (Grunlan et al., 2005; Rhim et al. 2006; Asharani et al., 2009; Jones and Hoek, 2010; Sintubin et al., 2011). Due to their small size, AgNPs have rapid diffusion, high specific surface area, and size similar to that of biomacromolecules. Silver species release Ag+ ions and they interact with the thiol groups in bacteria proteins affecting the replication of DNA and also collapse the proton-motive force across the cytoplasmic membrane (Lee et al., 2009). It is suggested that antibacterial activity can also occur through direct physical contact between the nanoparticles and bacterial cells, causing structural damage to their cell walls. Silver ions have been reported to interact with cytoplasmic components and nucleic acid to inhibit respiratory chain enzymes and to interfere with membrane permeability (Holt and Bard, 2005). The antibacterial activity depends on the silver ions, which bind strongly to electron donor groups on biological molecules containing sulfur, oxygen, phosphorus, or nitrogen atoms. As a result, it has been observed that bacterial death is due to the loss of the transport function of the bacterial cell membrane or DNA damage (Castanon et al., 2008). AgNPs have been immobilized on different organic-inorganic porous hosts (Tatar et al., 2007; Rivero et al., 2011). The researchers suggest that the important aspect of the hybrid matrix is that both organic and inorganic materials should not show any phase separation in order to get maximum homogeneity (Yano et al., 1998).
Statins and Alkylphospholipids as New Anticancer Agents Targeting Lipid Metabolism

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Abstract: The partial efficacy and high toxicity of the current anticancer chemotherapeutics as well as the development of multiple drug resistance are the major problems in cancer therapy. Therefore, there is a need for the development of novel well-tolerated anticancer agents with different mode of action that could be successfully used in combination with other drugs as an adjuvant therapy. The inhibition of intracellular signaling pathways associated with cancer growth and invasiveness is a main therapeutic approach in cancer treatment. It is well known that lipid metabolism is involved in the regulation of key cellular processes such as proliferation, differentiation and apoptosis. Statins and alkylphospholipids are both relatively new synthetic agents with considerable anticancer properties that disturb lipid metabolism and subsequently modulate proliferation and cell survival signaling pathways, leading to apoptosis. Numerous in vitro and in vivo studies have shown promising results for the use of statins and alkylphospholipids as potential therapeutic agents in the treatment of various human malignancies. However, more investigations and clinical trials are needed to assess their optimal safe dose and maximal efficacy and better understand the molecular mechanisms underlying the antitumor effects of these drugs.

Keywords: Statins, alkylphospholipids, lipid metabolism, intracellular signaling, antitumor potential.

INTRODUCTION

The development of novel target drugs with better efficacy and high selectivity which can be applied alone or in combination with conventional therapy is of utmost importance to improve the current cancer treatments and to overcome multiple drug resistance (MDR). Statins and alkylphospholipids (APLs) are both targeted agents interfering with lipid metabolism which appear very attractive drug candidates for the treatment of human hematological malignancies and solid tumors. Statins and alkylphospholipids constitute two different classes of drugs that have recently been shown to be highly effective for the treatment of a wide variety of tumor types. Statins, on first place, are drugs that decrease plasma cholesterol levels by an inhibition of HMG-CoA reductase and are the most effective medications for the treatment of hypercholesterolemia and reducing the risk of coronary heart disease [1,2]. APLs are synthetic lipids that initially were synthesized in the search for novel effective immune modulators and also have been shown to be efficacious for treatment of protozoan infections [3,4].

In the last few years the interest in statins and APLs has significantly increased due to the substantial evidence of their anti-proliferative, pro-apoptotic and anti-inflammatory properties to cancer cells, combined with a relatively low toxicity to normal cells and tissues [5]. Many reports have indicated that these drugs selectively induce apoptosis in tumor cells and simultaneously exhibit no/low cytotoxic effect in normal cells [6-11]. The exact molecular mechanism by which these two classes of drugs elicit their antitumor activity still remains unclear. It has been suggested that statins and APLs’ mode of action are primarily related to their interaction with cell membranes [12]. Both agents share a similar mechanism of action, disturbing functions of cell membranes and their components in a different way and subsequently affect signal transduction pathways. Statins interfere with the mevalonate pathway, an important biosynthetic process within lipid metabolism that is responsible for cholesterol synthesis [13], whereas alkylphospholipids affect mainly the synthesis of natural phospholipids, particularly phosphatidylcholine [3]. Recent studies have shown that alkylphospholipids affect not only the synthesis of phosphatidylcholine but also the synthesis and cell transport of cholesterol and synthesis sphingomyelin [14, 15]. Lipid metabolism plays a major role in the regulation of numerous cellular processes, including cell growth, proliferation, differentiation and motility [13]. Hence, the changes in lipid metabolism initiated by both types of drugs lead to inhibition of intracellular proliferative and survival signaling pathways and induction of apoptosis. It has been shown that statins as well as alkylphospholipids mainly suppress the both anti-apoptotic Ras/Raf/MEK/ERK and PI3K/Akt/MEK/MAPK pathways [16-19].

This review focuses mainly on the mechanism of action and antitumor effects of statins and alkylphospholipids against different tumor types from recent in vitro and in vivo studies, and clinical data.

Structure and Function of Cell Membranes

Cell membranes consist of various lipid species with different chemical properties including mono-, di- and triglycerides, fatty acids, glycerophospholipids, sphingolipids, sterol lipids and other compounds [20,21]. Lipids are structurally diverse water insoluble molecules that maintain cellular structure, provide energy and are involved in multiple cell signaling pathways [21,22]. Glycerophospholipids, sphingolipids and sterol lipids are the major structural components of eukaryotic membranes [21]. Plasma membrane lipids are asymmetrically distributed between the two membrane monolayers. The internal membrane leaflet is mainly

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Design and synthesis of gold-loaded micelles based on poly (ethylene glycol) and poly (4-vinyl pyridine) triblock copolymers for biomedical applications

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Abstract Gold-loaded micelles were prepared using triblock copolymers based on poly (ethylene glycol) and poly (4-vinyl pyridine) (P4VP-b-PEG-b-P4VP) obtained via atom transfer radical polymerization (ATRP). This was achieved using novel dichloro-terminated PEG as a macroinitiator. Further, ATRP was performed using 4-VP as a second forming block in the presence of CuCl/PMDETA as a catalyst system. The successful formation of P4VP-b-PEG-b-P4VP block copolymers was proved by FT-IR and ¹H NMR with the presence of all characteristic signals arising from PEG and 4-VP units. The self-assembling behavior of thus prepared block copolymer in an aqueous media was investigated. Further, gold nanoparticles were embedded into the P4VP core of the micelles. Cell viability of the gold-loaded micelles was proven by MTT test as they showed no cytotoxicity on cancerous and non-cancerous cells.

Keywords Block copolymers · Micelles · Gold nanoparticles · Cell viability

Introduction

The design and synthesis of nanostructured materials is one of the most attractive fields in the polymer science due to their potential applications in biomedicine, catalysis, optics, and so on [1–8]. Among them, great attention has been paid to polymeric micelles which can be used as nanoreactors for drug and gene delivery, metal, and semiconducting nanoparticles. Polymeric micelles are colloidal nanoparticles having an inner core and outer corona, which differs in polarity, and possess unique properties. They can be easily formed by self-assembly of amphiphilic polymers which can be obtained by controlled radical polymerization (CRP). This is a method which allows the synthesis of wide range of well-defined copolymers with controlled molecular weight and narrow molecular weight distribution. In general, CRP can be achieved by three main methods: nitroxide-mediated polymerization (NMP), atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer polymerization (RAFT) [9, 10].

Micelles for drug delivery can be used in many ways as one of them is to attach targeting ligands which specifically recognize and bind to receptors overexpressed in tumor cells. One of the exiting characteristic of polymeric micelles is their affinity toward metal nanoparticles. Much attention has been focused on core-shell metal nanoparticles based on gold, platinum, and quantum dots, because their properties differ significantly from their bulk state [11, 12]. In this regard, gold nanoparticles are well known with their optical properties originating from plasmons (surface plasmon resonance (SPR))—collective oscillations of highly mobile electrons resident in the metal at optical frequencies [13]. Gold nanoparticles have been used in catalysis, biological markers, display devices, DNA sensors, surface-enhanced Raman scattering, and cancer diagnostic and therapy [14, 15]. Attention has been paid to their possible application for photo-thermal therapy due to their strong and tunable linear absorption in the near-infrared (NIR) region where tissue penetration can be maximized. The biosafety of gold-based nanoparticles is currently
PVA-BASED HYBRID MATERIALS FOR IMMOBILIZATION OF *TRICHOSPORON CUTANEUM* R57 EFFICIENT IN REMOVAL OF CHROMIUM IONS

Nelly Georgieva, Rayna Bryaskova, Nevena Lazarova, Dimitar Peshev, Rumiana Tzoneva

*(Submitted by Corresponding Member A. Kossev on July 19, 2012)*

Abstract

New hybrid materials based on polyvinyl alcohol (PVA) and γ-aminopropytriethoxysilane (APTEOS), 3-mercaptopropyltriethoxysilane (MPTEOS) and tetraethoxysilane (TEOS) were prepared. To determine the thermal stability of the hybrid materials, thermogravimetric analysis (TGA) was performed. The new materials were tested for biofilm formation and biosorption studies. They were tested as matrices for immobilization of *Trichosporon cutaneum* R57 capable to remove Cr(VI) from aqueous solutions. The use of APTEOS and MPTEOS as precursors, incorporated new NH− and SH− bonds, which ensured additional binding places for cells immobilization. The presence of SH− and NH− groups in the hybrid matrices resulted in apparently higher Cr(VI) sorption capacity of the immobilized *Tr. cutaneum* R57 cells due to the incorporation of additional adsorption sites in the matrix through the PVA functionalities. The synthesized PVA/APTEOS materials proved efficient for use in biosorption applications.

**Key words:** polyvinyl alcohol, silica, *Trichosporon cutaneum* R57, chromium

1. Introduction. The chromium emissions and its high toxicity are major concerns in the development of new environmentally safety technologies. The search for new and innovative technology for the remediation of Cr(VI) pollution

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CYTOTOXICITY AND ANTIFUNGAL ACTIVITY
OF CMC/SiO₂/AgNps HYBRID MATERIALS AGAINST
SACCHAROMYCES CERESIUS 537


(Submitted by Corresponding Member Z. Lalchev on August 27, 2014)

Abstract

The paper focused on preparation of antimicrobial silica hybrid materials based on tetraethylorthosilicate (TEOS) as SiO₂ precursors, carboxymethyl cellulose (CMC) as an organic compound and silver (AgNps). The quantity of organic substance was 10 wt.% and the silver concentration varied from 0.0 to 1.5 wt.%. The obtained hybrids were analyzed and characterized using AFM analysis and the hydrophilicity of the materials was quantified by determining the water contact angle.

It has been experimentally demonstrated that these silver doped organic-inorganic hybrids have a well pronounced antimicrobial behaviour against Saccharomyces cerevisiae 537. The results showed that hybrid materials with 1.5 wt.% Ag resulted in 66.65% cell reduction after 24 h incubation. In addition the hybrid materials bearing AgNps were tested for their toxicity to 3T3 fibroblasts. Increasing the concentration of AgNps to 0.5 wt.% did not cause any decrease in cell survival; even after 48 h good cell proliferation was observed. With further increasing the AgNps to 1.5 wt.% and prolonged incubation (48 h) the cell viability drastically dropped down to 20%.

Key words: carboxymethyl cellulose, Saccharomyces cerevisiae 537, silver nanoparticles, sol-gel techniques, cytotoxicity, cell survival, proliferation

1. Introduction. Silver ions (Ag+) and their compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells [1-4]. Silver nanoparticles (AgNps) can directly damage bacteria cell membranes through release of silver ions followed (individually or in combination) by increased membrane permeability, loss of the proton motive force, inducing de-energization of the cells and efflux of phosphate,
THE INFLUENCE OF ANTI-CANCER AGENT ERUFOSINE ON GRAFFI MYELOID TUMOUR CELLS BEHAVIOUR.
CYTOTOXICITY AND CYTOSKELETON REORGANISATION STUDY

Veselina Uzunova, Sonia Apostolova, Ani Georgieva*, Martin R. Berger**, Reneta Toshkova*, Rumiana Tzoneva

(Submitted by Academician K. Koumanov on September 23, 2015)

Abstract

In the present study we aimed to evaluate the role of cytotoxicity of erufosine for eliciting changes in cytoskeleton organization and induction of apoptosis in Graffi myeloid tumour cells. The cytotoxicity of erufosine was revealed by MTT assay. The effect of erufosine on cytoskeleton and cell nuclei was evaluated by immunostaining for α-tubulin and F-actin, as well as by DAPI staining. We show that IC_{50} dose for EPC_{3} treatment of Graffi tumour cells was obtained at 20 μM. Fluorescent images showed existence of apoptosis at the same EPC_{3} concentration. The induction of apoptosis by EPC_{3} was accompanied by actin and tubulin reorganization. The obtained results revealed reorganization of actin cytoskeleton and induction of adhesive cell phenotype by erufosine treatment.

Key words: erufosine, reorganization of cytoskeleton, apoptosis, Graffi tumour cells

1. Introduction. Erufosine (EPC_{3}) is a novel chemotherapeutic agent belonging to a group of substances referred to as alkylphosphocholines (APCs).

This study was supported by grants DFNI-BO 02/5 and DNTS/Germany 04/01.
A NEW APPROACH USING NANOMEMBRANE-BASED THERAPEUTIC PLASMAPHERESIS FOR TREATMENT OF PATIENTS WITH MULTIPLE SCLEROSIS AND NEUROMYELITIS OPTICA


(Submitted on November 24, 2015)

Abstract

A number of neurological diseases are related to autoimmune pathogenesis. These are multiple sclerosis, Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy, monoclonal gammopathy, myasthenia, polymyositis, and others. The mentioned diseases are characterized by autoantibodies from immunocompetent cells connected to myelin of the central and peripheral nervous system, the neuromuscular junction, calcium channels, etc.

According to Guidelines on the use of therapeutic apheresis in clinical practice-evidence-based approach from the writing committee of the American Society for Apheresis: the sixth special issue, over 75 diseases can be treated with therapeutic apheresis, including the aforementioned neurological diseases.

We have introduced for the first time in Bulgaria a qualitatively new method of therapeutic apheresis. Basic features: (1) Use of nanotechnology

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This research was supported by Grant No B-02/20/2014 from the National Science Fund, Bulgaria.
EFFECT OF ELECTRICAL FIELD AND MILTEFOSINE ON ACTIN AND ACTIN-ASSOCIATED PROTEIN ZO-1 ORGANIZATION IN CANCER AND NON CANCEROUS CELLS

Aneliya Kostadinova, Irina Georgieva, Tanya Topouzova-Hristova*, Rumiana Tzoneva

(Submitted by Academician K. Koumanov on February 29, 2016)

Abstract

The actin cytoskeleton plays a key role in the stability of cell-cell junctions, adhesion and cell motility, which are crucial for tumour progression and metastases. Changes in the actin filaments organization could affect and increase permeability of cell monolayer due to interruption and reorganization of tight junctions in epithelial cells.

In this study we investigate the combined effect of the electroporation and miltefosine on the F-actin and ZO-1 in cancer epithelial line A549 and non-cancer cell line MDCKII. Our results showed that treatment of A549 and MDCK cells with electrical field in combination with miltefosine is cell-specific. The cancer A549 cell line was found to be more sensitive to the treatment as compared to non-cancerous cells. Actin cytoskeleton was highly disturbed while ZO-1 organization seems stabilized.

Key words: miltefosine, electrical pulses, actin, ZO-1

Introduction. One of the major challenges facing modern medico-biological science is the search for new anti-cancer drugs and therapies. Recently, the new branch of synthetic alkylphospholipids (ALPs) was introduced [1]. Unlike conventional anticancer agents, which act predominantly at DNA level, ALPs act on the cellular membrane, because of their similarity to the endogenous phospholipids. At low concentrations ALPs are inserted into the plasma membrane and subsequently cause a wide range of biological responses that ultimately lead
HPC HYBRID HYDROGELS WITH EMBEDDED SILVER NANOPARTICLES FOR ANTIBACTERIAL SCAFFOLDS. BIOCOMPATIBILITY TESTING

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(Submitted by Academician K. Koumanov on February 19, 2016)

Abstract

The biocompatibility of hybrid hydroxypropyl cellulose (HPC) hydrogels with different content of embedded silver nanoparticles (AgNPs) is revealed by testing the cytotoxicity and induction of cell death. The effect of cytotoxicity and cell death was studied on L929 fibroblasts by using MTT cytotoxicity assay, cell morphological observations and acridine orange/ethidium bromide (AO/EtBr) live cell staining. The results showed good biocompatibility of hydrogels containing AgNPs in the range 0.5-1.5 wt % Ag. Cells incubated with HPC hydrogels with the higher amount of AgNPs (2-2.5 wt % Ag) presented morphological changes corresponding to cell death and increased grade of cytotoxicity revealed by MTT assay. All these data suggest that AgNPs content in HPC materials exhibits dose-dependent threshold over which the biocompatibility of the hydrogels is disturbed. Hybrid materials with low silver content – 0.5 wt % to 1.5 wt % Ag proved their biocompatibility and will be suitable candidates for biomedical applications.

Key words: antibacterial materials, silver nanoparticles, biocompatibility, cytotoxicity, cell death

Introduction. Nowadays, considerable attention is focused on silver nanoparticles (AgNPs) because of their unique chemical and physical properties and pronounced antibacterial activity, which provide one of the most cost effective alternatives for the development of new antibacterial materials. It is well known that silver and silver ions are effective antimicrobial agents having an activity against bacteria, viruses and fungi [1, 2]. Free AgNPs were shown to be cytotoxic

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Hemocyanin from *Rapana thomasiana* – structure and anti-breast cancer activity in a presence of cholinium amino acids

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**Abstract**

The focus of the present research is a hemocyanin, isolated from the hemolymph of marine snails *Rapana thomasiana* (RtH), and its interactions with ionic liquids, based on a cholinium cation and a non-polar amino acid as an anion [Chol][AA]. Six RtH-[Chol][AA] complexes have been obtained. Fragmentation and structural changes in the protein, even at low protein to ionic liquid ratios, were observed using static light scattering and fluorescence spectroscopy. For the first time the cytotoxicity of RtH, [Chol][AA] and their complexes on adenoma breast cancer MDA-MB-231 cells was tested. All protein-ionic liquid complexes reduced moderately the cell viability of MDA-MB-231 cells, however, the effect was weaker than those estimated for the pure RtH or [Chol][AA].
PIECES OF TAKE VENETO NA BEFOLE VENETO ADHERENT TUMORNI II SOMATICNI KLETKI. VLIYASIE NA ELEKTRONIZACIYA NA BKYRU KLETCHNATNA PUTOCKSCHNOST.

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ABSTRACT

Cell adhesion plays a key role in tumor progression and its control could diminish the tumor metastases. In the present study the action of eurrosine on reorganization of actin cytoskeleton and apoptosis was analyzed in breast cancer and mammary epithelial cells. The influence of electroporation on cytotoxicity was studied also.

Breast cancer cell lines MDA-MB-231 and MCF-7 as well as non-transformed MCF-10A were treated with eurrosine (5-15mM) or subjected to combine treatment with biphasic electrical pulses. MTS test, actin and DAPI staining were used.

For MDA-MB-231 eurrosine provoked apoptosis and actin reorganization, since MCF-7 and MCF-10A were less sensitive to the action of eurrosine. The combine treatment with eurrosine and electrical pulses lead to stimulation of cell proliferation for MCF-7 and lack of additional effect for MDA-MB-231.

The cytotoxic action of eurrosine on breast cancer cells and epithelial cells is cell specific. The most sensitive is the high invasive MDA-MB-231 cell line since 15 mM eurrosine cause cytoskeleton reorganization and apoptosis.

Keywords: eurrosine, breast cancer cells, apoptosis, actin cytoskeleton

Увод


Клетъчната адхезия е фундаментален процес който при нетрансформираните клетки играе ключова роля за клетъчния растеж и преживяемост, тъй като участва в организацията на тъканите и органите [Hynes R., 2002]. При трансформираните (ракови) клетки, наличието на адгезивни контакти не е условие за растеж и преживяемост [Fish S. and Rnosalviti E., 1997], тъй като основните особености на раковите клетки са намалената
ABSTRACT

Breast cancer is a disease with a high metastatic potential, and in many cases leads to a resistance to applied standard anti-cancer drugs. This necessitates to looking for more effective anti-tumor agents and combined approaches for cancer therapy. The action of erufosine and electrical field on cell survival, reorganization of cytoskeleton, and cell cycle was analyzed in breast cancer and mammary epithelial cells. Breast cancer cell lines MDA-MB-231 and MCF-7 as well as the non-tumorigenic line MCF-10A were treated with erufosine (5 µM to 50 µM) and electrical field (500 V/cm and 100V/cm). MTS test, FACS analysis and actin staining were performed. In MDA-MB-231 cells erufosine in combination with the electric field induces a significant reduction in cell survival and depolymerization of the actin cytoskeleton. There are also changes in the cell cycle of treated MDA-MB-231 cells, which is an increase of G2/M phase, indicating the existence of mitotic cell arrest. MCF-7 and MCF-10A are less sensitive to the combined treatment. Application of electrical pulses has an additive effect on enhancing the antitumor activity of erufosine. The most sensitive to the combined treatment with erufosine and electric field is highly invasive cell line MDA-MB-231.

Keywords: erufosine, electric field, actin cytoskeleton, cell cycle

УВОД. Алкилфосфосфолипидите (АФЛ) са нова група анти-туморни агенти, които показват цитотоксична активност срещу различни туморни клетъчни линии in vivo и антитуморна активност in vitro. За различия от класическите цитостатики, които действат на ниво ДНК (антиракундрици, таксани, антибактериални, цисплатин и др.), алкилфосфолипидите проявяват своеето действие на ниво клетъчна мембрана. Голямо разнообразие от цитотоксични механизми са приписани на алкилфосфолипидните компоненти. Сред тях са промени на протеините, включително и фосфолипидите, инхибирани на фосфатидилхолинова синтеза, активиране на SARK/TNK сигнален път и др. Наскоро се синтезират нов алкилфосфолипиев препарат, наречен eucyphospho-N. N, N-trimethylpropylammonium. Агентът може да стимулира продукцията на хематопоетичните прегенераторни клетки. Показано е, че еруфозинът индуцира аполоза и автофагия чрез модулиране на Akt-mTOR сигнализа в път. Клетъчната адхезия е фундаментален процес, който в натуморните клетки играе ключова роля в клетъчните растеж и поддържа организацията на тъканите и органите. При туморните клетки присъствието на адхезионни контакти не е предпоставка за растеж и диференциране. Промените в клетъчната адхезия определят модифицираната им морфология и миграция, което е свързано с техните инвазивни свойства по време на всички стадии на туморното развитие. По този начин манипулирането на клетъчната адхезия е жизнена предпоставка за контролиране на туморния растеж и инвазия. Основна роля в клетъчната адхезия играе активност цитоскелета. Нашите предхождащи изследвания показваха, че електрически импулси предизвикват промени в клетъчната адхезия и цитоскелетна организация, а еруфозинът причинява клетъчна смърт и провокира адхерентен клетъчен фенотип. Досега нямаш данни за комбинираното действие на

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Influence of electric field on cell behavior. Electrotreatment of cells for biomedical applications

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Electric fields are present in all developing and regenerating animal tissues. During recent years, there has been growing research on the response of cells to the endogenous and exogenously applied electric field. This review discusses the role of endogenous and applied (exogenous) electric field mainly in stimulating tissue regeneration and in cancer treatment by describing: 1) the historical context of bioelectricity, 2) the fundamental principles of cell and tissue response to electric fields, 3) the cellular mechanisms for the effects of low and high electric fields on cell behavior, and 4) the perspectives of using new techniques and approaches for electric field application in tissue regeneration and cancer treatment. © Anita Publications. All rights reserved.

Endogenous electric field

Direct-current (DC) electric fields (EFs) are present in all developing and regenerating tissues and have a potential impact on tissue repair and development. In the 18th century, Galvani, one of the most famous imitators of modern electrophysiology, performed his significant experiment on frog nerve-muscle preparations [1, 2] (Fig. 1). He used different electric stimuli (generated from lightning storms from static electric discharge to cause leg muscles to twitch. In his experiment he provided strong evidence for “animal electricity” using stimulated contractions of frog sciatic nerves. Galvani had demonstrated for the first time the existence of the injury potential (Fig. 1A). The injury potential is the steady, long-lasting DC voltage gradient induced within the extracellular and intracellular spaces by current flowing into and around an injured nerve [2]. In contrast, the action potential is a rapid, self-regenerating voltage change localized across the cell membrane. It was demonstrated for the first time in nerve and muscle by Matenucci in the 20th century using the injury potential of damaged frog muscle. By placing a cut nerve into an injured muscle, the latter activated the nerve (Fig. 1A), causing a contraction of the innervated muscle. In the past two centuries, the relative importance of injury potentials and action potentials has shifted markedly. Since action potentials have become of a major importance in neuroscience and electrophysiology, injury potentials have acquired much more attention in regenerative medicine in recent years. For instance, injury currents, like those discovered by Galvani, were proven to play a role in nerve regeneration [3]. The injury currents have been measured by entering the cut ends of Mauthner and Muller axons into embryonic lamprey spinal cords (Fig. 1B), which are of the order of 100 µA/cm², and because the resistivity of soft tissues is ~1,000 Ω·cm, they give rise to steady voltage gradients of ~10 mV/mm. In this way, the injury potential, which is established by these currents in the distal ends of cut axons, impedes regeneration [4]. In the last 20 years, this work has progressed to human clinical trials using applied DCEF's to treat human spinal cord injuries.

A good example of the injury currents was shown in skin. Human skin and that of guinea pigs and amphibians maintain a transepithelial potential (TEP) across the epithelial layers. How does this happen? Individual cells maintain an electric potential (V_m) across the intact plasma membrane (Fig. 1C). This is a result of the activity of membrane bound channels. These channels are selective for the transport of specific ions across the intact membrane, which has a high electric resistance. Such selectivity causes the formation of a net negative charge on inside of the cell relative to the outside. The selective directional ion transport across intact epithelia results in a significant potential difference across the epithelial layer (Fig 1D). Those principles are valid to all ion-transporting epithelia, including multilayered epithelia, such as mammalian skin and the corneal epithelium. For instance, Xenopus skin scavenges Na⁺ from the dilute pond water and pumps within epithelial cell membranes. The apical part of the cell, which faces the pond

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ANTIFUNGAL EFFECT OF SILVER DOPED HYBRID MATERIALS BASED ON SILICA AND CARBOXYMETHYL CELLULOSE AGAINST ASPERGILLUS NIGER

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Abstract
The aim of the present work was investigation the antifungal activity of sol-gel derived hybrid materials against Aspergillus niger. The hybrids were prepared from tetraethyl orthosilicate (TEOS), carboxymethyl cellulose (CMC) and silver nitrate. The quantity of silver was 0.5 and 1.5 wt. %. In this research were used two antifungal methods – one based on measuring size of inhibition zones and the second one included the ability of the materials to inhibit fungi growth on the agar surface in contact with it. The antifungal properties of materials against eukaryotic cells depend on the silver concentration. Both assays revealed that the hybrids have a restraining impact on A. niger and slowed down its growth. The composites prepared using metal nanoparticles and polymers can be more effective due to their enhanced antimicrobial activity.
IN VITRO ANTIPROLIFERATIVE ACTIVITY OF THE NOVEL ANTI-CANCER AGENT ERUFOSINE ON GRAFFI MYELOID TUMOR CELLS

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Abstract

Alkylphosphocholines (APCs) are synthetic phospholipid analogues with a pronounced antineoplastic activity mediated by an interference with the lipid metabolism of the tumor cells and modulation of cellular signaling pathways regulating the proliferation, differentiation and apoptosis.

In the present study the antiproliferative activity of the novel APCs erucylphospho-N, N, N-trimethylpropanolamine (erufosine) on in vitro cultured Graffi myeloid tumor cells was determined by a (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) (MTT) dye reduction assay. The dose- and time-dependence of the antineoplastic effect was assessed after exposure to four different concentrations of erufosine. The tested anti-cancer agent was found to exert a clear dose-dependent antiproliferative effect on Graffi myeloid tumor cells. The IC₅₀ value of the erufosine was calculated. The morphological changes in the Graffi tumor cells cultured in the presence of erufosine were analyzed by live/dead staining with acridine orange and ethidium bromide and were visualized by by ZOE™ Fluorescent Cell Imager. The microscopic observations support the claim that the erufosine causes death of Graffi tumor cells through induction of apoptosis. Based on these results we could suggest that the erufosine appears to be a promising agent in the treatment of haematological malignances.

Key words: alkylphosphocholines, erufosine, Graffi myeloid tumor cells, antiproliferative activity
In vivo antitumor effect of the novel alkylphosphocholine erufosine applied alone or in combination with doxorubicin against Graffi myeloid tumor in hamsters

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Abstract
Erufosine belongs to the group of alkylphosphocholines (APCs), which are novel synthetic membrane-targeting anticancer agents. APCs have attracted the scientific interest not only with their pronounced antineoplastic properties, but also with the ability to increase the efficacy of chemotherapy and radiotherapy in vitro and in animal experiments.
In the present study, the in vivo antitumor effect of erufosine applied alone or in combination with the conventional chemotherapeutic drug doxorubicin was assessed in hamsters with experimental Graffi myeloid tumor. The results demonstrate the protective antitumor effect of erufosine and doxorubicin, expressed by reduction of the transplantability, tumor growth inhibition, decreased mortality and extension of the mean survival time. These effects were most clearly pronounced in the experimental groups with a combined treatment. The presented results suggest that the combined application of doxorubicin and erufosine could be a promising antitumor treatment strategy. Further studies are needed to clarify the biological and therapeutic effects of these substances using different experimental conditions. The results obtained may be used for the purposes of the medical oncotherapeutic practice.

Key words: erufosine, alkylphosphocholines, Doxorubicin, Graffi myeloid tumor
PREPARATION OF AMPHIPHI LIC PDMS BLOCK COPOLYMER
NANOMATERIALS CONTAINING PVP FOR BIOMEDICAL APPLICATION

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ABSTRACT

In this study, we present PDMS-based amphiphilic block copolymers containing PVP (Poly N-vinyl pyrrolidone) hydrophilic polymer block with different chain length. The amphiphilic copolymers are synthetized by conventional radical polymerization initiated with rarely used PDMS-macrono initiator. From these block copolymers we prepared nanocontainers (polymersomes) for drug delivery application, accomplished by film rehydration, solvent evaporation and extrusion methods and nano ranged 3D amphiphilic polymer scaffolds, using versatile technique such as electrospinning. PDMS-based amphiphilic block copolymers containing PVP (Poly N-vinyl pyrrolidone) were studied for biological response: cell proliferation, cell adhesion and morphology after contact with polymer surfaces. The obtained materials are biocompatible, due to the optimal chain length of hydrophilic blocks, pore size and roughness of polymer materials based of polymersomes. All materials are characterized by different spectroscopic and microscopic techniques.

Key words: PDMS, PVP, macrono initiator (MAI), diblock copolymer, SEM, XPS, DLS, polymeric vesicles, electrospinning, biocompatibility, drug delivery