Резюмета на публикациите на английски език

на гл. асистент д-р Соня Николова Апостолова,

кандидат за участие в конкурс за заемане на академична длъжност "доцент" в област на висше образование 4. Природни науки, математика и информатика, професионално направление 4.3. Биологически науки, научна специалност „Биофизика", за нуждите на лаборатория "Трансмембранна сигнализация" към Институт по биофизика и биомедицинско инженерство, Българска академия на науките.

Група от показатели В

Показател В4: Научни публикации в издания, които са реферирани и индексирани в световноизвестни бази данни с научна информация (Web of Science и Scopus)

1. **[B4.1] Modification of Rapana thomasiana hemocyanin with choline amino acid salts significantly enhances its antiproliferative activity against MCF-7 human breast cancer cells**

   Maya Guncheva, Krasimira Paunova, Paula Ossowicz, Zbigniew Rozwadowski, Ewa Janus, Krassimira Idakieva, Svetla Todinova, Yuliana Raynova, Veselina Uzunova, **Sonia Apostolova**, Rumiana Tzoneva and Denitsa Yancheva

   Abstract

   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 [Chol][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–[Chol][AA] on breast cancer cells (MCF-7) and 3T3 fibroblast cells (non cancerous) was evaluated. We found that all [Chol][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 [Chol][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–[Chol][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.

2. [B4.2] Rapana thomasiana hemocyanin modified with ionic liquids with enhanced anti breast cancer activity

Maya Guncheva, Krasimira Paunova, Paula Ossowicz, Zbigniew Rozwadowski, Ewa Janus, Krassimira Idakieva, Svetla Todinova, Yuliana Raynova, Veselina Uzunova, **Sonia Apostolova**, Rumiana Tzoneva, Denitsa Yancheva

Abstract

This is the first study on the surface modification of a hemocyanin from marine snail Rapana thomasiana (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 antiprofilerative activity of 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 24h) 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.

3. [B4.3] Effects of riboflavin on hyperalgesia and serum glutamine-to-glutamate ratio in rats with painful diabetic neuropathy

Milen Hristov, Zafer Sabit, Tsvetomir Kirilov, Dimitar Bakalov, Rumiana Tzoneva, **Sonia Apostolova**, Irina Georgieva, Pavlina Andreeva-Gateva

Abstract

Previous studies have explored the antinociceptive effects of riboflavin (vitamin B2) across various experimental models. However, there remains a gap in the literature regarding its potential to alleviate neuropathic pain in diabetes. This study aims to investigate the effects of riboflavin on hyperalgesia and serum glutamine-to-glutamate ratio in rats with painful diabetic neuropathy. In fasted rats, a model of painful diabetic neuropathy was induced through intraperitoneal injection of streptozotocin. In the fifth week post-injection, diabetic rats experiencing neuropathic pain were administered daily doses of riboflavin (25 or 50 mg), dissolved in their drinking water, for a duration of two weeks. Results demonstrate that riboflavin significantly reduced mechanical and cold-induced hyperalgesia in diabetic rats compared to controls. Formalin-induced hyperalgesia was alleviated by riboflavin in the second phase. Additionally, riboflavin supplementation increased the serum glutamine-to-glutamate

ratio in these animals. These findings highlight the therapeutic potential of riboflavin in managing neuropathic pain associated with diabetes.

4. **[B4.4] 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

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 ?-tubulin and F-actin, as well as by DAPI staining. We show that IC 50 dose for EPC3 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.

5. **[B4.5] HPC Hybrid Hydrogels with Embedded Silver Nanoparticles for Antibacterial Scaffolds. Biocompatibility Testing**

Uzunova V., **Apostolova S**., Angelova Ts., Toshkova R., Georgieva N., Tzoneva R.

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 exibits 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.

Група от показатели Г

Показател Г7: Научни публикации в издания, които са реферирани и индексирани в световноизвестни бази данни с научна информация (Web of Science и Scopus)

1. **[Г7.1] Tuning of the Anti-Breast Cancer Activity of Betulinic Acid via Its Conversion to Ionic Liquids**

   Paula Ossowicz-Rupniewska, Joanna Klebeko, Irina Georgieva, **Sonia Apostolova**, Łukasz Struk, Svetla Todinova, Rumiana Dimitrova Tzoneva, Maya Guncheva

   Abstract

   Betulinic acid (BA) is a natural pentacyclic triterpene with diverse biological activities. However, its low water solubility limits its pharmaceutical application. The conversion of pharmaceutically active molecules into ionic liquids (ILs) is a promising strategy to improve their physicochemical properties, stability, and/or potency. Here, we report the synthesis and characterization of 15 novel ILs containing a cation ethyl ester of a polar, non-polar, or charged amino acid [AAOEt] and an anion BA. Except for [ValOEt][BA], we observed preserved or up to 2-fold enhanced cytotoxicity toward hormone-dependent breast cancer cells MCF-7. The estimated IC50 (72 h) values within the series varied between 4.8 and 25.7 μM. We found that the most cytotoxic IL, [LysOEt][BA]2, reduced clonogenic efficiency to 20% compared to that of BA. In addition, we evaluated the effect of a 72 h treatment with BA or [LysOEt][BA]2, the most cytotoxic compound, on the thermodynamic behavior of MCF-7 cells. Based on our data, we suggest that the charged amino acid lysine included in the novel ILs provokes cytotoxicity by a mechanism involving alteration in membrane lipid organization, which could be accompanied by modulation of the visco-elastic properties of the cytoplasm.

2. **[Г7.2] Antinociceptive Behavior, Glutamine/Glutamate, and Neopterin in Early-Stage Streptozotocin-Induced Diabetic Neuropathy in Liraglutide-Treated Mice under a Standard or Enriched Environment**

   Pavlina Gateva, Milen Hristov, Natasha Ivanova, Debora Vasileva, Alexandrina Ivanova, Zafer Sabit , Todor Bogdanov, **Sonia Apostolova**, Rumiana Tzoneva

   Abstract

   Diabetic neuropathy (DN) is a common complication of long-lasting type 1 and type 2 diabetes, with no curative treatment available. Here, we tested the effect of the incretin mimetic liraglutide in DN in mice with early-stage type 1 diabetes bred in a standard laboratory or enriched environment. With a single i.p. injection of streptozotocin 150 mg/kg, we induced murine diabetes. Liraglutide (0.4 mg/kg once daily, i.p. for ten days since the eighth post-streptozotocin day) failed to decrease the glycemia in the diabetic mice; however, it alleviated their antinociceptive behavior, as tested with formalin. The second phase of the formalin test had significantly lower results in liraglutide-treated mice reared in the enriched environment vs.

liraglutide-treated mice under standard conditions [2.00 (0.00-11.00) vs. 29.00 (2.25-41.50) s, p = 0.016]. Liraglutide treatment, however, decreased the threshold of reactivity in the von Fray test. A significantly higher neopterin level was demonstrated in the diabetic control group compared to treatment-naïve controls and the liraglutide-treated diabetic mice ($p < 0.001$). The glutamine/glutamate ratio in both liraglutide-treated groups, either reared under standard conditions ($p = 0.003$) or an enriched environment ($p = 0.002$), was significantly higher than in the diabetic controls. This study demonstrates an early liraglutide effect on pain sensation in two streptozotocin-induced diabetes mouse models by reducing some inflammatory and oxidative stress parameters.

3. [Г7.3] Pinealectomy-Induced Melatonin Deficiency Exerts Age-Specific Effects on Sphingolipid Turnover in Rats

Jane Tchekalarova, Irina Georgieva, Teodora Vukova, **Sonia Apostolova**, Rumiana Tzoneva

Abstract

The existing body of literature, in conjunction with our recent studies, shows that melatonin dysfunction can accelerate the aging process, with this effect depending on the specific age of the subject. The present study aims to ascertain the impact of pinealectomy on sphingolipid (SL) turnover in young adult (3-month-old), middle-aged (14-month-old), and old (18-month-old) rats. Ceramide (Cer) levels, neutral (NSMase) and acid sphingomyelinase (ASMase), acid ceramidase (ASAH1), and sphingosine-1-phosphate (S1P) levels in hippocampus and/or plasma, were evaluated by enzyme-linked immunosorbent assay. The accumulation of Cer and its metabolite second messenger S1P in the hippocampus and plasma was associated with increased levels and activity of hippocampal NSMase in the hippocampus and plasma. However, no such association was observed for hippocampal ASMase, whose levels and activity were reduced in middle-aged and old rats compared to young adult rats. Pinealectomy-induced melatonin deficiency in young adult rats showed an increase in hippocampal Cer content compared to the sham group. However, in contrast to young adult rats, pinealectomy had an inverse effect on age-related changes in hippocampal Cer, NSMase, and ASMase in middle-aged rats. Furthermore, pinealectomy exacerbated the age-related increase in S1P in the hippocampus of 18-month-old rats. Collectively, the results of the present study suggest that melatonin deficiency may influence the aging process by modulating SL turnover in an age-specific manner.

4. [Г7.4] Novel Fluorescent Benzimidazole-Hydrazone-Loaded Micellar Carriers for Controlled Release: Impact on Cell Toxicity, Nuclear and Microtubule Alterations in Breast Cancer Cells

Rayna Bryaskova, Nikolai Georgiev, Nikoleta Philipova, Ventsislav Bakov, Kameliya Anichina, Maria Argirova, **Sonia Apostolova**, Irina Georgieva, Rumiana Tzoneva

Abstract

Fluorescent micellar carriers with controlled release of a novel anticancer drug were developed to enable intracellular imaging and cancer treatment simultaneously. The nanosized fluorescent micellar systems were embedded with a novel anticancer drug via the self-assembling behavior of well-defined block copolymers based on amphiphilic poly(acrylic acid)-block-poly(n-butyl acrylate) (PAA-b-PnBA) copolymer obtained by Atom Transfer Radical Polymerization (ATRP) and hydrophobic anticancer benzimidazole-hydrazone drug (BzH). Through this method, well-defined nanosized fluorescent micelles were obtained consisting of a hydrophilic PAA shell and a hydrophobic PnBA core embedded with the BzH drug due to the hydrophobic interactions, thus reaching very high encapsulation efficiency. The size, morphology, and fluorescent properties of blank and drug-loaded micelles were investigated using dynamic light scattering (DLS), transmission electron microscopy (TEM), and fluorescent spectroscopy, respectively. Additionally, after 72 h of incubation, drug-loaded micelles released 3.25 µM of BzH, which was spectrophotometrically determined. The BzH drug-loaded micelles were found to exhibit enhanced antiproliferative and cytotoxic effects on MDA-MB-231 cells, with long-lasting effects on microtubule organization, with apoptotic alterations and preferential localization in the perinuclear space of cancer cells. In contrast, the antitumor effect of BzH alone or incorporated in micelles on non-cancerous cells MCF-10A was relatively weak.

5. [Γ7.5] *In Vitro* Cytotoxicity Assessment of Betulinic Acid Organic Salts on Triple-Negative Breast Cancer Cells

Sonia Apostolova, Irina Georgieva, Paula Ossowicz-Rupniewska, Joanna Klebeko, Svetla Todinova, Rumiana Tzoneva, and Maya Guncheva

Abstract

The conversion of betulinic acid (BA) to organic salts is a strategic approach to modulate its physicochemical properties and biological activity. In our previous study, we demonstrated the enhanced cytotoxicity of certain amino acid ethyl ester betulinates ([AAOEt][BA]) compared to BA against hormone-dependent breast cancer cells (MCF-7). In this study, we extended our investigation to evaluate the cytotoxic response and thermodynamic properties of hormone-independent breast cancer cells (MDA-MB-231) following 72 h of treatment with the same series of betulinates. Our data reveal a lower cytotoxic response in MDA-MB-231 cells, indicated by higher half-maximal inhibitory concentration (IC_{50}) values, which ranged between 31 and 109 µM. Differential scanning calorimetry analysis supported these findings, showing negligible changes in the thermodynamic parameters of the treated MDA-MB-231 cells. However, consistent with our previous observations, [LysOEt][BA]2, exhibited the highest cytotoxicity and induced the most pronounced morphological alterations in the cancer cells. Overall, our results suggest that MDA-MB-231 cells are less sensitive to [AAOEt][BA] compared to MCF-7 cells, likely due to their distinct phenotypic and genotypic profiles and differences in oncogenic signalling pathways. Nonetheless, the fact that [LysOEt][BA]2 enhances the cytotoxic activity of BA even in hormone-independent breast cancer cells underscores its therapeutic potential and warrants further investigation, particularly in the context of adjuvant breast cancer therapy.

6. [Г7.6] Phenolic Content, Antioxidant Activity and *In Vitro* Anti-Inflammatory and Antitumor Potential of Selected Bulgarian Propolis Samples

Yulian Tumbarski, Ivan Ivanov, Mina Todorova, **Sonia Apostolova**, Rumiana Tzoneva, Krastena Nikolova

Abstract

Background/objectives: Propolis (bee glue) is a valuable bee product widely used as a natural remedy, a cosmetic ingredient, a nutritional value enhancer and a food biopreservative. The present research aims to investigate the phenolic content, antioxidant activity and *in vitro* anti-inflammatory and antitumor potential of six propolis samples from three regions of Bulgaria (Vidin, Gabrovo and Lovech).

Methods: the analysis of propolis phenolic compounds was determined by high-performance liquid chromatography (HPLC); the antioxidant activity of ethanolic propolis extracts was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric-reducing antioxidant power (FRAP) assay; the in vitro anti-inflammatory activity was assessed by the inhibition of albumin denaturation method; the *in vitro* antitumor activity was determined in human metastatic breast cancer cell line MDA-MB-231 using 3-(4,5-Dimethyl -2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay.

Results: The ethanolic propolis extracts exhibited the total phenolic content from 190.4 to 317.0 mg GAE/g, total flavonoid content from 53.4 to 79.3 mg QE/g and total caffeic acid derivatives content from 5.9 to 12.1 mg CAE/g. The studied propolis extracts showed significant antioxidant capacity (between 1000.3 and 1606.0 mM TE/g determined by the DPPH assay, and between 634.1 and 1134.5 mM TE/g determined by the FRAP assay). The chemical composition analysis indicated high concentrations of caffeic acid benzyl ester, chrysin, pinocembrin and pinobanksin-3-O-propionate, predominantly in three of the propolis samples originating from Gabrovo and Lovech regions. All propolis samples demonstrated promising *in vitro* anti-inflammatory activity, expressed as the inhibition of thermally induced albumin denaturation by 73.59% to 78.44%, which was higher than that of the conventional anti-inflammatory drugs Aspirin (58.44%) and Prednisolone Cortico (57.34%). The propolis samples exhibited high *in vitro* cytotoxicity against cancer cells MDA-MB-231 with IC_{50} values ranging between 9.24 and 13.62 μg/mL as determined by MTT assay.

Conclusions: Overall, we can suggest that the high phenolic content of Bulgarian propolis from respective areas contributes to its enhanced antioxidant, anti-inflammatory and antitumor activity. Taken together, our results support the beneficial properties of Bulgarian propolis, with potential application as a promising therapeutic agent.

7. [Г7.7] A Standardized Extract of *Petasites hybridus* L., Containing the Active Ingredients Petasins, Acts as a Pro-Oxidant and Triggers Apoptosis through Elevating of NF-κB in a Highly Invasive Human Breast Cancer Cell Line

Sonia Apostolova, Tsvetelina Oreshkova, Veselina Uzunova, Irina Georgieva, Liliana Maslenkova, Rumiana Tzoneva

Abstract

Background: Common butterbur (*Petasites hybridus* L.) is a traditional medicinal plant with numerous therapeutic properties among which is its recently uncovered anti-tumor activity. The present study aims to examine the activity of a standardized Bulgarian Petasites hybridus L. root extract, containing the active ingredients petasins, on the human breast cancer cell line MDA-MB-231 and non-cancerous MCF-10A cells. Specifically, we examined cell death, oxidative stress, and nuclear factor kappa-B (NF-κB) signaling.
Methods: A standardized butterbur powdered extract containing a minimum of 15% petasins was used. A lipophilic extract was obtained from subterranean portion of the plant of Bulgarian populations of *Petasites hybridus* using liquid-liquid extraction after completely removing pyrrolizidine alkaloids. The induction of apoptosis and necrosis was analyzed by flow cytometry, and oxidative stress biomarkers and NF-κB were measured using enzyme-linked immunosorbent assay (ELISA).
Results: *Petasites hybridus* L. root extract triggered apoptosis in a cancer-specific fashion and induced a moderate oxidative stress characterized by diminished glutathione (GSH) levels and elevated malondialdehyde (MDA) levels in MDA-MB-231 72 h after treatment. NF-κB levels were higher in cancer cells after treatment with IC_{50} and IC_{75} doses, this suggested that the NF-κB pathway was activated in response to oxidative stress leading to the induction of apoptosis. MCF-10A cells were affected to a lesser extent by the *Petasites hybridus* extract, and the adaptive response of their antioxidant defense system halted oxidative stress.
Conclusions: Overall, these results indicate that *Petasites hybridus* L. root extract selectively acts as a pro-oxidant in breast cancer cells and thus represents a potential therapeutic option for cancer treatment with fewer side effects.

8. [Г7.8] Statins and Alkylphospholipids as New Anticancer Agents Targeting Lipid Metabolism

Sonia N Apostolova, Reneta A Toshkova, Albena B Momchilova, Rumiana D Tzoneva

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 an emergency 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.

9. [Г7.9] Angiogenic potential of endothelial and tumor cells seeded on gelatin-based hydrogels in response to electrical stimulations

Rumiana Tzoneva, Veselina Uzunova, **Sonia Apostolova**, Anne Krüger-Genge, Axel T Neffe, Friedrich Jung, Andreas Lendlein

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 MMP's production after the application of EF. Gelatin-based matrices with 3 (G10_LNCO3), 5 (G10_LNCO5), 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-26TM) were used. For an estimation of the amount of VEGF released from cells a commercially available VEGF ELISA (Thermo Fisher 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.

10. [Г7.10] Testing biocompatibility in terms of cell viability and nuclei alterations of materials for the development of artificial hip joints

Alexander Gerchev, **Sonia Apostolova**, Irina Georgieva, Vanya Milanova, Veselina Uzunova, Rumiana Tzoneva

Abstract

In this study we evaluated the *in vitro* biocompatibility of different materials which were undergone mechanical processing and were used for the fabrication of artificial hip joints. The MTT assay was used as quantitative analysis to assess cell viability after treatment of murine fibroblast L929 cell line with extracts of Ti6Al4V, Stainless steel and UHMWPE materials. The impact of implant materials on nuclear morphology was analyzed by DAPI staining. We have shown that all tested materials exhibit high biocompatibility and non-toxicity after 72 h and 5 days of incubation. This was confirmed by the analysis of cell nuclei, showing no alterations of the nuclear shape and size after 72 h and 5 days, respectively. Furthermore, dividing cells have been observed in the extracts of the three different materials, indicating that cell proliferation is not affected and corresponding implant materials allow normal cell growth. Our results demonstrate that all studied materials are biocompatible and non-toxic to fibroblasts and therefore could be successfully used as orthopedic implants for hip joint replacement.