Scientific contribution/Original research

Biomechanics of Joints at 6th Socratic Lectures

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Abstract:
Traditionally, Socratic Lectures are mainly devoted to students and young scientists. To optimize the transfer of knowledge from excellence to youth, curricula and examination procedures were being updated since the initiation of this process in 2008. This year, 8 students of Medicine taking place at the Faculty of Medicine, University of Ljubljana have participated in the elective subject Biomechanics of joints. Within this contribution we report on the subject and the examination process and results.

Keywords: Extracellular vesicles; Neuropathy; Brachycephaly; Stem cells; Microalgae; Nanoplastics

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1. Curriculum

In the academic year 2021/2022, the elective subject Biomechanics of joints took part of the winter semester. The lectures were organized in the second half of November followed by analysis of the biomechanical parameters of hips and Socratic Lectures with the examination. The lecturers were prof. Vane Antolič, prof. Matej Drobnič and the leading author of this contribution. Due to positive experience with online teaching from 2020/2021, we decided to continue with online curricula also in the future, regardless of the COVID-19. Namely, a significant part of the subject is devoted to the HIPSTRESS method for determination of biomechanical parameters of hips (resultant hip force and distribution of contact hip stress). For that, digitized X-ray images are used and analyzed by an appropriate graphical software. It is convenient for the students to immediately use the software during the lectures and follow the method by screen sharing. Furthermore, the number of students that chose the subject this year (8) allowed individual consultations which increased the quality of the analysis made by the students. This year, the students continued with the analysis of a series of periacetabular osteotomies which started a year ago. To calculate biomechanical parameters, a new HIPSTRESS model for resultant hip force is being tested (Uršič et al., 2021). The new model is dimensionless at the expense of simplification to only one effective muscle. This simplification has been introduced because the newer digitized X ray images do not contain a unit length, therefore scaling of reference muscle attachment points that was included in the original 3-dimensional HIPSTRESS model (Iglič et al., 1993) could lead to large errors. Each student analyzed 8 – 12 X ray images. In testing the method, all participants simultaneously analyzed the same X ray image and inserted the measurements as well as calculations in a common google drive excel document. This allowed for immediate checking of the results and finding the errors in formulas and measurements. Furthermore, the students inserted analysis of the X-ray images directly to the excel document which made the averaging over the populations fast and easy. The student Jan Zmazek has previously graduated from the Faculty of Physics and is presently also a PhD student. As he is skilled with computer as well as with mathematical modeling, he introduced his own improvements in the method by using the software Geogebra and inserting in it the solution of a nonlinear algebraic equation which is obligatory to determine the hip stress distribution. The students cooperated successfully between each other to share the skills and contribute the knowledge that they acquired previously.

2. Examination

The examination questions were sent to the students a day before. A group of questions addressed the plenary lecture. As there were 7 scientific sections and 8 students, each student was assigned to one section and the corresponding questions. The students were advised to get acknowledged with the subjects and to ask the participants of the symposium for help in answering the questions. All tools and social networks were allowed to be used for answering the questions. The questions were posed according to the state of the art in the respective fields; therefore, no decisive answers are yet available, and the students were obliged to consider the available arguments and take a standpoint. This symposium's plenary lecture was given by prof. Antonella Bongiovanni from Palermo, Italy, on green extracellular vesicles which are being considered as carriers of substances in medical applications. The scientific sections covered the fields of medicine, veterinary medicine, orthopedics, biomaterials, ecology, physics, and extracellular vesicles. After the end of the scientific section the students have assembled the answers into one MS Word document and have submitted it to an official mail of the Socratic Lectures within the limited time.

3. Results

Below we present the revised version of the document created by the students in which the answers to the questions were sought.

3.1. What are extracellular vesicles (EVs)?

EVs are sub-micron sized membrane-enclosed entities shed or secreted by cells that cannot replicate (Théry et al., 2018). They can be shed from the plasma membrane or from internal departments of the cell. They are called microvesicles and exosomes, respectively (**Figure 1**)

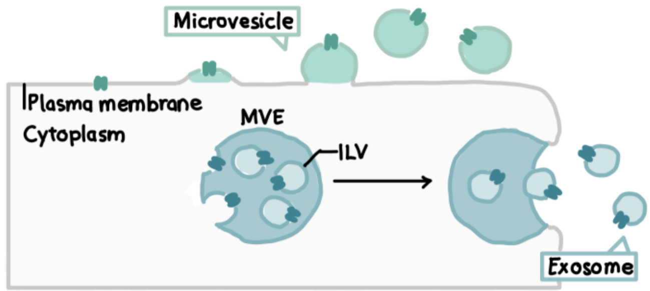

Figure 1. Scheme of microvesicle and exosome shedding. Adapted from van Niel et al. (2018).

EVs can be isolated in vitro from cultured media or from different biological samples (blood, urine, saliva, cerebrospinal fluid, tissue, wastewater). EVs are intrinsic mediators of intercellular communication in the body, allowing functional transfer of biomolecules (lipids, proteins, DNA, mRNA, microRNAs, and other noncoding RNA) between diverse locations (Yáñez-Mó et al., 2015). EVs have diverse biologic roles in different tissues as biomarkers for different diseases and as potential therapeutic agents (Svenson, 2012, Yáñez-Mó et al., 2015, Fais et al., 2016). They are involved in the modulation of genetically encoded messages via miRNA trafficking (Ullah et al., 2019) and in regeneration of tissue (Taverna et al., 2017). Due to these pleiotropic effects, EVs are hypothesized to play an influential role in modulating the tissue microenvironment as it relates to the repair and regeneration of damaged or diseased tissues, as well as for the removal of unwanted proteins and toxic materials (Ullah et al., 2019).

The role of EVs in different pathophysiological conditions (cancer, cardiovascular disease, infectious diseases, and neurodegenerative disorders) is being investigated (Ullah et al, 2019). It was suggested that EVs may act in synergy with bone marrow-derived stem cells in inflammation, immunomodulation, and cellular reprogramming, and that they interact with stem cells and cancer cells in the process of oncogenesis (Ullah et al., 2019). The advantages of the EV delivery compared to liposome delivery include simpler technical manufacturing and delivery and reduced cost (Gepstein et al., 2019, Çağdaş et al., 2021).

3.2. How are EVs identified?

EVs can be identified based on size, morphology, shape, membrane presence, structure, proteins, density, topology, etc. (Adamo et al., 2021). The size of plant vesicles is between 30 and 150 nanometers in diameter (Bongiovanni, 2021). Experimental methods for EV identification (pointing to their origin and mechanism of formation) are electron microscopy and diverse omics (Kralj-Iglič et al., 2020). Furthermore, EVs are characterized by immunoblotting, density determination by gUC, fluorescamine assays, stability testing, testing resistance to detergents, light scattering, flow cytometry, different spectrometric assays and nanotracking analysis (Adamo et al., 2021).

3.3. How are EVs experimentally distinguished from viruses?

Taking into account definitions, viruses can take over cellular pathways to replicate inside the cell while EVs cannot (Théry et al., 2018; Bongiovanni, 2021). However, in encapsulated viruses such as SARS-CoV-2 the cargo is enclosed within a bilayer-based membrane (Bongiovanni, 2021), similarly to EVs. Such virions structurally resemble EVs, which makes the distinction between them difficult. It was found that retroviruses resemble EVs in size and density (50-100 nm, 1.13–1.18 g/L) (Nolte-'t Hoen et al., 2016). In a HIV study, separation of EVs from HIV virions was based on faster migration of virions than EVs in velocity gradients (Nolte-'t Hoen et al., 2016, Lazarian et al., 2018). Discrimination of EVs from virions included determination of capsid protein p24 in virions and of acetylcholinesterase and lymphocyte common antigen (CD45, CD stands for cluster of differentiation) in EVs (Nolte-'t Hoen et al., 2016). HIV virions were considered not to incorporate acetylcholinesterase and CD45 in their membrane, however, it is unclear whether these molecules are carried by all EVs (Nolte-'t Hoen et al., 2016; Cantin et al., 2008).

3.4. Which are the biological roles of EVs?

Biological roles of EVs are to maintain cellular and tissue homeostasis by transferring critical biological cargos to distal or neighboring recipient cells (Zarà et al., 2011). Because they participate in the pathogenesis of several diseases they can be used as disease biomarkers, as well as therapeutic tools in tissue regeneration and novel options for drug delivery (Zarà et al., 2011).

3.5. How could EVs be used for drug delivery inside the organism?

Drug delivery mechanisms of EVs are currently being investigated for their capability of carrying lipids, proteins, and nucleic acids throughout the human body (Bongiovanni, 2021). Cells communicate with each other by exchanging information through the secretion of soluble factors such as growth agents, cytokines, and genetic material, all of which can be encapsulated within EVs (Ullah et al., 2019).

3.6. Describe the mechanism by which EVs could deliver drugs to recipient cells?

Constituents of the EV membrane can interact with receptors that are present in the plasma membrane of recipient cells (Zaborowski et al., 2015). In addition, EVs were found to be internalized into recipient cells via different endocytic pathways that are characteristic for different cell types (Zaborowski et al., 2015). Endocytosis is the process by which cells take in substances from outside of the cell by engulfing them in a vesicle (Gleichmann, 2020). These can include things like nutrients to support the cell or pathogens that immune cells engulf and destroy (Gleichmann, 2020). EVs can be loaded with drugs after being isolated or during their formation (Elsharkasy et al., 2020).

3.7. From which sources could EVs be harvested for wide use drug delivery?

EVs can be harvested from different human cells (kidney, muscle, cardiac, liver, intestinal, nerve, epithelial, cancer, dendritic, immune and stem cells), bacteria, plants, animals (bovine milk) and microalgae. However, human cells are difficult to obtain in large enough quantities to be widely used, thus the recent fascination with microalgae, which are abundant and show promising results.

3.8. Which methods would be appropriate to produce a sufficient amount of EVs for these means?

Various methods have been developed to isolate EVs from microalgae-conditioned media, differing in yield, purity, and size distribution of isolated EVs (Kang et al., 2017). Nanoalgosomes were harvested by differential ultracentrifugation, gradient ultracentrifugation, and tangential flow filtration (Adamo et al., 2021). In ultracentrifugation the centrifuge rotor attains centripetal accelerations over 100.000 g, where g = 10 m/s² (Adamo et al., 2021). Differential centrifugation consists of consecutive centrifugation steps to separate particles with different sizes, while in gradient centrifugation, liquids with different densities are poured on top of each other to collect the particles with matching densities (Adamo et al., 2021). In tangential flow filtration the culture samples pass the filters embedded in cassettes to collect EVs which are further concentrated (Adamo et al., 2021).

3.9. Describe at least one possible mechanism of clinical use of EVs.

Mesenchymal stem cell EVs have high potential for bone and tissue regeneration due to a combination of pro-angiogenic, anti-apoptotic and immunomodulatory factors (Marangon, 2021). While there are preclinical studies using bone marrow MSC to treat bone defects in rats and mice (Marangon, 2021), it is the possibility of isolating EVs from different biofluids that makes them valuable biomarkers to be analyzed for the diagnosis or prognosis of several conditions. Their complex cargo reflects the (patho)physiologic status of the cells from which they originate. Moreover, natural nanoparticles have been investigated as therapeutic tools in many pathological conditions (Ciferri et al., 2021). The methods for harvesting EVs include limitations in the process of conventional invasive tissue biopsies, because the procedure can lead to infection and cannot be used on all tissues. Instead, the minimally invasive 'liquid biopsy' is a better strategy (Allelein et al., 2021).

3.10.Which are the limitations of the methods for harvesting and characterization of EVs?

During centrifugation, filtration, and passage through chromatographic columns, EVs are subjected to shear stresses which may change their morphology and composition or even cause their destruction or aggregation. According to Juares (2021), during processing, EVs may get contaminated by proteins and soluble factors; polymer-based precipitation can lead to co-precipitation of protein contaminants and polymeric materials and immunoaffinity capture-based techniques induce antibody cross-reactivity. Static/dynamic light scattering, tunable resistive pulse sensing and nanoparticle tracking methods can be inaccurate with poly-dispersed and size heterogeneous samples which is often the case with isolates (Chiriacò et al., 2018). Flow cytometry has a detection limit (>100 nm, flow cytometer dependent), a swarming effect (identification of multiple vesicles as a single event) and cannot distinguish EVs from other particles of similar size (i.e., protein/antibody aggregates) (Chiriacò et al., 2018). ELISA/Western Blot can also detect non-EV proteins (Chiriacò et al., 2018). Atomic force microscopy has poor resolution, classical scanning electron microscopy requires fixation and metal sputtering which causes EV shrinking (Chiriacò et al., 2018). The majority of these methods were developed for other systems, e.g., cells or inorganic nanoparticles and are only partially suitable for tiny fragile particles with transient identity (such as EVs).

3.10. What is neuropathy and which are its biomechanical effects?

Peripheral neuropathy is described as damage or dysfunction of one or more nerves within the peripheral nervous system (Cleveland Clinic, 2019). The presented symptoms depend on the type of nerve (sensory, motor, autonomic including digestion and circulation) that is damaged; the most common risk factors include diabetes, trauma, meta-

bolic syndrome, alcoholism, abnormal vitamin levels, particular medications and poisons, cancer treatment with chemotherapy, some inherited disorders, autoimmune disorders, and infections (Cleveland Clinic, 2019). The common signs and symptoms are tingling or numbness (usually in the hands and feet - distal sensory loss that spreads proximally), sharp, burning pain, changes in sensation, falling, loss of coordination, muscle weakness, twitching, cramps, spasms and/or paralysis (Cleveland Clinic, 2019). The symptoms can appear suddenly (acute neuropathy) or develop slowly over time (chronic neuropathy) (Schara, 2021). Distal symmetric polyneuropathy is a chronic complication of diabetes mellitus with prevalence of 6.3% – 50% (Mankowsky, 2021). It usually starts with lesions on peripheral sensitive nerves and progresses to motor and autonomic nerves (Sartor et al., 2012). It causes progressive loss of vibratory, thermal, tactile, and proprioceptive sensitivities (Sartor et al., 2012). Muscle atrophy, musculoskeletal impairments, and autonomic dysfunction can be established in later stages of the disease, mainly due to impairment of the larger diameter neural fibers (Sartor et al., 2012).
The biomechanical effects of neuropathy are limitation of mobility of the foot and ankle joints, alterations in spatial-temporal patterns of gait (velocity, step length, stride length, and time of double support) in delayed leg and thigh muscle activation, resulting in unfavorable alterations of ground reaction forces, and plantar pressure during gait (Sartor et al., 2012). These alterations may lead to foot ulcerations and increase risk for foot amputation (Mankowsky, 2021). The best options for minimizing these problems are wound debridement, dressing and off-loading. Most common and effective tools for off-loading are crutches, healing shoes and total contact cast (Schara, 2021). They reliably reduce plantar pressure, increase healing rate, and time in healing and reduce complications (Schara, 2021).

3.11. Which are the biomechanical effects of wearing the above knee leg prosthesis?

The most common reasons for leg amputations are illness (tumor, infection, frostbite, cardiovascular diseases, diabetes) and accidents (Ipavec, 2021). The above knee prosthesis involves an artificial knee which can be mechanical (based on hydraulic system or locking mechanism) or microprocessor-assisted (Ipavec, 2021). The important parameters are the height of the prosthesis, the prosthetic socket (required comfortable fit) and the prosthetic foot (corresponding to the type of artificial knee and to the shape of artificial heel) (Ipavec, 2021). An above knee prosthesis can have a significant impact on gait, with substantial deviations of symmetry, step length, hip exertion and upper body involvement (Ipavec, 2021). These differences can produce gait that is less efficient and less comfortable, resulting in slower and shorter walking distance (Ipavec, 2021). Other problems that can appear with an above knee amputation and wearing of the prosthesis are phantom pain, poor fit of the prosthetic socket, blisters, tissue thickening on the amputee's residual limb and allergic reactions to the prosthetic socket's material (Ipavec, 2021).

3.12. Which are the biomechanical effects of brachycephaly in dogs? How can brachycephaly affect homeostasis and hemostasis in dogs?

In dogs, brachycephaly causes the shortening of the muzzle, flat facial conformation and widening of the skull, without a corresponding decrease in the size of the soft tissue in the skull (**Figure 2**).

Brachycephaly is perceived as a desirable trait in domesticated dogs, with commonly encountered breeds being English bulldog, French bulldog, pug, Boston terrier, Shih tzu, etc. (Šimundić, 2021). Brachycephaly in dogs causes elongated soft palate, macroglossia, stenotic nares, undersized nasal chambers, malformed and aberrantly growing nasal conchae, and tracheal hypoplasia (Šimundić, 2021). Consequent respiratory signs include dyspnea, stertor, stridor, increased respiratory effort, exercise intolerance, heat, or stress intolerance and even cyanosis and collapse (Žgank et al., 2021). Biomechanical effect of brachycephaly in dogs is difficulty in breathing, mainly caused by the airway obstruction by soft tissue rather than tracheal diameter (Žgank et al., 2021).

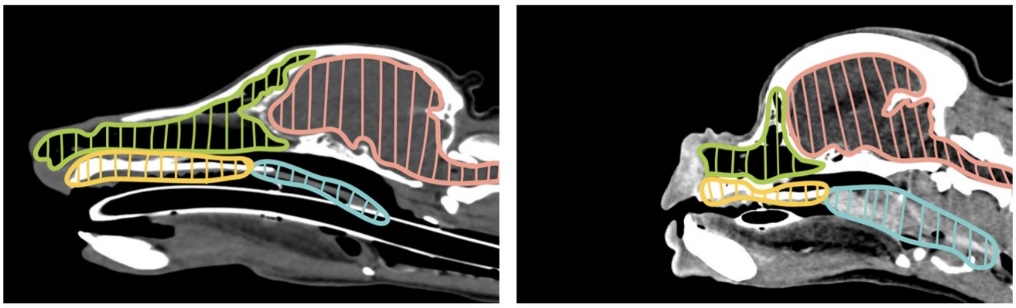

Figure 2. Magnetic resonance image of the cross section of the canine skull. Left: dolichocephalic dog, right: brachycephalic dog. The shape of the elements is marked by shading. Soft tissue is marked by cyan color (Erjavec and Lukanc, 2021).

The homeostasis and hemostasis effects of brachycephaly in dogs are polycythemia, hypercoagulative state, hypomagnesemia, oxidative stress, myocardial damage, and respiratory acidosis (Erjavec and Nemec, 2021). Compensatory sinus tachycardia with weak pulse occurs due to respiratory insufficiency (Smajlović, 2021). The tachypnea is also observed due to laryngeal paralysis, edema, and airway obstruction (Smajlović, 2021). Inflammation might lead to disseminated intravascular coagulation (Smajlović, 2021). Laboratory evaluations also show that brachycephalic breeds are more prone to hemoconcentration (Smajlović, 2021).

3.13.How can we identify and characterize stem cells?

According to reviews by Hongxiang et al. (2011), stem cells are cells with capability of self-renewal and potential to differentiate into many types of cells in the body. There are two main types of stem cells: embryonic stem cells, derived from embryonic blastocysts, and adult stem cells, found among specialized cells within adult tissues or organs (Hongxiang et al., 2011). Embryonic cells are stem cells with full potential to differentiate, they are pluripotent (Hongxiang et al., 2011). Adult stem cells are only partially differentiated stem cells that appear to have a more restricted ability of producing different cell types and self-renewing, they are multipotent (Hongxiang et al., 2011). Adult stem cells are found throughout the body after embryonic development in organs and tissues such as brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, etc. (Hongxiang et al., 2011). According to their decent, adult stem cells can be hematopoietic, endothelial, olfactory, neural crest, testicular, mammary, neural, and mesenchymal stem cells (Spasovski, 2021). Adipocytes develop from mesenchymal cells and their differentiation is a complex process of events accompanied by changes in cell morphology, hormone sensitivity and gene expression (Bunnell et al., 2008). Stromal cells that have preadipocyte characteristics can be isolated from adipose tissue of adult subjects in large or small quantities, propagated in vitro and induced to differentiate into adipocytes (Bunnell et al., 2008). The term "Adipose-derived Stem Cells" (ASCs) identifies the isolated, plastic-adherent, multipotent cell population (Bunnell et al., 2008). Methods to isolate these cells from the adipose tissue, start with the collection of adipose tissue by needle biopsy or liposuction aspiration (Bunnell et al., 2008). ASCs can then be isolated by washing the tissue sample extensively with phosphate-buffered saline (PBS) containing 5% Penicillin/Streptomycin (P/S) (Bunnell et al., 2008).

3.14.Describe at least one possible mechanism of treatment with stem cells.

Stem cells are used in regenerative medicine in the process of creating living, functional tissues to repair or replace tissue or organ whose function is lost due to several conditions like disease, aging, damage, or congenital defects (Perez-Terzic et al., 2014). The potential use of stem cells in medical therapy is widespread and it can be used to cure various diseases such as blindness, deafness, myocardial infarction, muscular dystrophy, diabetes, Crohn’s disease, different types of cancer, spinal cord injury, wound healing, stroke, missing teeth and neurodegenerative diseases, osteoarthritis, and others (Spasovski, 2021). Adipose tissue – derived stem cells are being used for treatment of osteoarthritis (Spasovski, 2021). Samples of fat tissue are first obtained by surgical excision and then processed in vitro, including isolation, propagation, harvesting and phenotyping (Spasovski, 2021). After 15-24 days of growth, the cells are applied to the joint by needle injection (Spasovski, 2021).

3.15.How can the presence of endoprosthesis in the body affect homeostasis and hemostasis?

Table 1. Information related to the key essential metals which are used for different medical implants (U.S Food and Drug Administration, 2019).

Metal	Major Physiological Roles of Proteins Utilizing the Metal	Key Manifestation of Deficiency	Potential Toxicities or Manifestations of Excess
Cobalt (Co)	Metabolism of purines/ pyrimidines, amino acids, fatty acids, folate	Anemia, Neuropathy, Neurocognition changes	ACD*, Cardiomyopathy, Polycythemia, Altered thyroid function
Copper (Cu)	Collagen cross-linking, Bone formation, Iron metabolism, Hemostasis/thrombosis, Neurotransmitter synthesis	Iron-refractory anemia, Neutropenia/infection, Osteoporosis, Neurological dysfunction	GI symptoms, Hemolysis, Cardiac failure, Renal failure, Hepatic dysfunction, Alzheimer’s
Iron (Fe)	Oxygen transport/storage, DNA synthesis/repair, RNA transcription, Synthesis of collagen, Immune function	Microcytic anemia, Diminished thyroid function, Impaired neutrophil function, Impaired cognition	Free radical generation, GI symptoms (acute), Hemochromatosis: Cardiomyopathy, Cirrhosis, Diabetes, Arthritis
Manganese (Mn)	Metabolism of carbohydrates, lipids, urea, Neurotransmitter synthesis, Bone/cartilage formation	Dermatitis, Weight loss, Growth retardation, Abnormal bone/cartilage, Dyslipidemia, Glucose intolerance	Headache, Psychiatric symptoms, GI symptoms, Parkinson’s-like signs/symptoms
Molybdenum (Mo)	Metabolism of nucleotides, amino acids, neurotransmitters	Urinary tract stones, Acute renal failure, Myositis, Mental changes/coma	Elevated uric acid/gout, Secondary copper deficiency, Reduced testosterone
Zinc (Zn)	Immune function, Wound healing, DNA synthesis and repair, Stabilization of protein structure, Intracellular signaling	Decreased immune function, Delayed wound healing and growth, Neurological and bleeding abnormalities, Osteoporosis	Copper deficiency, Myeloneuropathy
Chromium (Cr)	Glucose metabolism/tolerance, Lipid metabolism	Impaired glucose tolerance, Abnormal lipids profiles, Peripheral neuropathy	Cr3+: Potential liver issues and kidney issues, CR6+: Respiratory and GI symptoms, Dermatitis/ulcerations, Lung cancer
Vanadium (V)	Phosphate metabolism, Insulin enhancement, Lipid metabolism	/	GI symptoms, Headache, Weakness, Tremor

*Abbreviations: ACD: Allergic contact dermatitis; GI: Gastrointestinal; UA: Uric Acid.

The foreign body reaction is an unavoidable process which takes place whenever any material becomes implanted into the body (Carnicer-Lombarte et al., 2021). The process of implantation injures the tissue around the foreign object, which triggers an inflammatory process) (Carnicer-Lombarte et al., 2021). Over a period of weeks to months this inflammatory process develops into a fibrotic response, which envelops and isolates the implanted material (Ullah et al., 2019). When the foreign material is implanted with the aim of delivering a therapy, both the acute (predominantly inflammatory) and chronic (fibrotic) stages of the foreign body reaction pose significant challenges to its integrity and therapeutic function (Ullah et al., 2019). Myeloid cells such as neutrophils and macrophages are the primary cells involved in the expected acute inflammation with subsequent peri-implant wound healing (Ullah et al., 2019). An implant may continue to elicit a chronic inflammatory response, lasting for months or longer and is characterized by a broader immune cell infiltration including both myeloid and lymphoid cells (Ullah et al., 2019). Chronic inflammation by implanted metal devices or metal wear debris may lead to adverse clinical effects (**Table 1**).

3.16.Describe at least one possible mechanism for maintenance of homeostasis of the ecosystem which is based on microalgae.

Microalgae are ecologically important photosynthetic organisms. They inhabit a highly diverse range of habitats (sea ice, sea waters, snow, inland waters, and soil) (Yong et al., 2016). As carbon fixators, primary producers in food chains and food sources for higher trophic organisms, microalgae play a crucial role in maintaining the equilibrium of food webs in the aquatic ecosystem (Yong et al., 2016). Climate change could affect the growth of microalgae, which could have a negative impact on the ecosystem homeostasis (Yong et al., 2016). Moreover, microalgae are sources of materials for biotechnological applications and medical use (Yong et al., 2016).

3.17.What are bisphenols and which are the mechanisms of their translocation between water, air, and soil?

Bisphenols are a group of chemicals that have been used for manufacturing plastics, epoxy resins and other products since the 1960s (Vehar, 2021). Bisphenols can enter the environment either directly from chemical, plastic and staining manufacturers, paper or material recycling companies, enterprises, which use bisphenols in casting sand, or indirectly leaching from plastic, paper, and metal waste in landfills (Vehar, 2021). The mechanism of translocation between water, air and soil mainly depends on bisphenol physical and chemical characteristics (Vehar, 2021). Water soluble bisphenols are more often found in water, those with lower density are more likely to pollute the air (Vehar, 2021).

3.18.Which are biological effects of micro and nanoplastics on cells?

The understanding of the biologic effects of micro - and nanoplastics on cells and the corresponding toxicity mechanisms is still limited. Human cell-based studies provide fundamental and valuable information on the key toxicity mechanisms. In general, toxicity mechanisms of micro - and nanoplastics depend on their size, surface characteristics, polymer type, as well as cell type (Matthews et al., 2021). The toxicity of micro - and nanoplastics on human cells is mainly attributed to nanoplastic particles, since microplastics cannot enter the cells due to their size (Banerjee et al., 2021). Toxicity mechanisms mainly include membrane disruption, extracellular polymeric substance disruption, reactive oxygen species generation, DNA damage, cell pore blockage, lysosome destabilization, and mitochondrial depolarization (Banerjee et al., 2021).

3.19.Describe the photo effect. Some models describe light as particles and some models describe it as a wave. How can we decide which model to use?

The photoelectric effect (**Figure 3**) is a phenomenon in which electrically charged particles are released from or within a material when it absorbs electromagnetic radiation (Encyclopedia Britannica, 2021). The effect is often defined as the emission of photoelectrons from a metal surface when exposed to light (Encyclopedia Britannica, 2021). One inexplicable observation is that the maximum kinetic energy of the emitted photoelectrons does not vary with intensity of the light or duration of exposure (above a certain frequency that is needed for dislodgement of the electrons), as expected according to the wave theory, but is instead proportional to the frequency of light (Encyclopedia Britannica, 2021). However, light intensity did determine the number of electrons released from the metal - measured as an electric current (Encyclopedia Britannica, 2021). That is why Albert Einstein proposed a particle model of light, which describes a beam of light as a swarm of discrete energy packets, known as photons and features particle properties such as momentum and energy (Encyclopedia Britannica, 2021). Such an approach is used when discussing the interaction of light with atoms and molecules, however it cannot explain diffraction, interference and the doppler effect, which are all wave-like phenomena (Cannata, 2021). The wave model of light on the other hand operates with wavelength and frequency and is used in macroscopic phenomena, optometry, and optical instruments (Cannata, 2021).

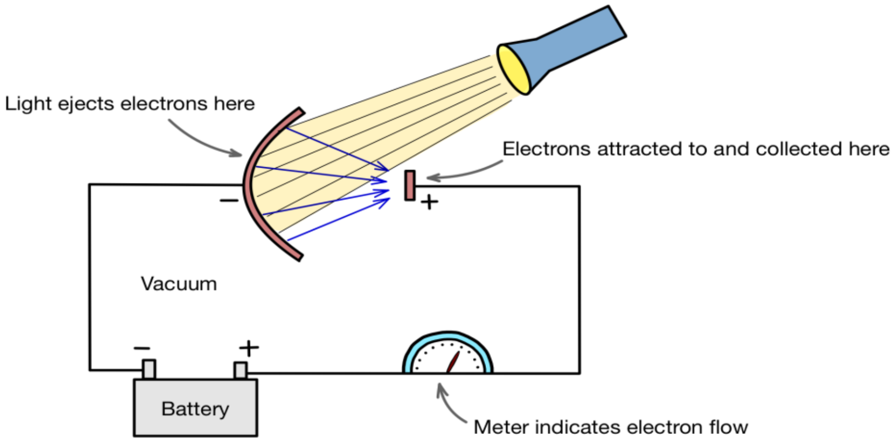

Figure 3. A scheme of the photoelectric effect. Adapted from (Cannata, 2021).

3.20.What was in your opinion the role of Mileva Marič in formulation of the theory of relativity, statistical physics, and quantum mechanics?

Mileva Marič worked closely with Albert Einstein, as she was a mathematician, a physicist, his partner and later his wife (Cannata, 2021). There is no definite proof of her contributions to the theory of relativity, statistical physics, and quantum mechanics, however in his letters to her Einstein refers to his work as “ours” (Cannata, 2021). Some sources also claim Mileva’s name was on the first paper about relativity and that as a mathematician she worked on the mathematical basis of Albert’s theories, took notes from foreign literature, and wrote scientific essays (Cannata, 2021). Einstein has never acknowledged Mileva’s help, but it seems like she was a collaborator to his work during a crucial, however difficult, time in history when women were socially discriminated against and often signed under acknowledgments instead of being co-authors of papers (Cannata, 2021).

3.21.Can you see a connection between black holes and membranous nanostructures?

Black holes are objects characterized by so much gravity pull that even light cannot get away from them (Mesarec, 2021). They are like membranous nanostructures in topological sense, as relativity is all about curvatures, only more complex and in more dimensions than membranes (Mesarec, 2021). Physics and calculations, for example membrane curvature tensors, in both examples are so akin, that we can use these nanostructures as a playground for describing black holes (Mesarec, 2021). Furthermore, the theory of membranes from a mathematical point of view, when applied to more dimensions, can be one of the simplest ways of describing string theory and curvatures of space-time (Mesarec, 2021).

4. Conclusions

It is our intention to continuously improve and update the method, the curriculum, and the examination process to initiate the students in the research process and immediately enable their active involvement in creating new knowledge. Also, it is important that the students enter the scientific society and acknowledge the values of the state of the art. The most important contribution to this is their decision to devote their professional life to consider the emergent problems and support that they provided to the lecturers of the symposium.

Conflicts of Interest: The authors declare no conflict of interest.

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