Szakmai zárójelentés a NKFIH által támogatott, 115259 azonosító számú, "Funkcionális biokompatibilis és biodegradábilis gélek szintézise és orvosi alkalmazhatóságának vizsgálata" című kutatásról 2015-09-01 - 2020-08-31-ig terjedő időszakra.

In order to imitate the structure of the backbone of the connective tissue, electro-spinning technique was applied to prepare artificial extracellular matrix. To prevent the polymer fibers from dissolution poly(succinimide) molecules were grafted by thiol side chains. During the electro-spinning process at 10 kV (0.8 ml/h flow rate and 15 cm target distance) cross-linking reaction took place between the thiol side chains. The mean value and distribution of the fibre diameter were determined with AFM and light microscope after the sample preparation. In vitro biocompatibility test with human fibrosarcoma (HT1080) and human fibroblast cells was performed. We have successfully modified the poly(succinimide) polymer with RGD peptide sequence, which was confirmed with 1H-NMR measurements. Polymer-hydrogels could be synthesized from these polymers and after a mild hydrolysis (close to the physiological conditions) the polymers transformed to poly(aspartic acid).The novel biocompatible and biodegradable artificial scaffold seems to be a promising poly(amino acid) based fiber matrix for tissue replacement.

We have successfully synthesized poly(aspartic acid) based hydrogels with different chemical constitutions (RGD side chain and thiol groups) to increase cell viability on the gel surface. The results show that higher the thiol groups concentration higher the amount of the viable cell number on the gels. The multiphoton microscopic images show that the cells were able to migrate in to the gel matrix and create a pre-tissue structure. The differentiation ability of the cells was investigated and we have found that the cells showed spontaneous differentiation activity on the high thiol containing gels.

We have created artificial scaffold with a fibre diameter in the nanometer range, loaded with silver nanoparticles. The size of the silver nanoparticles was determined with dynamic light scattering. The synthetized particles were polydispers (Zave: 2±1nm; PDI:0.5) and we could prepare 2D and 3D scaffolds in the presence of silver particles.

A continuous electrospinning method for gel fiber preparation was presented without a spinning window. We have used a special setup: a coaxial needle. As proof of

concept, the preparation of poly(aspartic acid)-based hydrogel fibers and their properties were described by using poly(succinimide) as shell polymer and 2,2,4(2,4,4)-trimethyl-1,6-hexanediamine as cross-linker in the core of the nozzle. Cross-linking took place as the two solutions get in contact at the tip of the nozzle.

Different poly(aspartamide)-dopamine conjugates were prepared and their physical and chemical properties were investigated. The solubility and the lipophilicity of the conjugates were measured. The release of dopamine from the conjugates was monitored by UV-VIS spectroscopy. The kinetics of dopamine from the macromolecular prodrugs having good water solubility has been studied and compared in different environments (phosphate buffer, Bromelain and a-Chymotrypsin). It was found that the kinetics of release in those solutions can be satisfactorily described by first order reaction rate. For poorly-soluble conjugates, the release of dopamine was considered as a result of coupling of diffusion and chemical reaction. Besides the time dependence of dopamine cleavage, a practical quantity, the half–life of the release of loading capacity has been introduced and evaluated. It was found, that dopamine containing macromolecular prodrugs exhibit prolonged release kinetics and the quantitative description of the kinetics, including the most important physical parameters provides a solid base for future pharmaceutical and medical studies.

We have studied the viability and osteogenic differentiation capacity of periodontal ligament stem cells (PDLSCs) cultured on poly(aspartamide) (PASP)-based hydrogels having different thiol group densities. The PDLSCs, originated from impacted human wisdom teeth were seeded on the PASP-based hydrogels possessing different mechanical and chemical properties. Multipotent mesenchymal stem cells derived from tooth-associated tissues have strong regeneration potential for several tissue types development of novel scaffolds that provide suitable three dimensional (3D) environment for survival and growth of these cells. In order to visualize the cells growing into the gels, fluorescent vital dye Vybrant DiD and a two-photon microscope were applied. Cell viability analysis was carried out using WST-1 reagent. Poly(aspartic acid) based hydrogels proved to be considerably biocompatible and biodegradable. Furthermore, these hydrogels did not only support survival but adhesion, proliferation, and migration of human PDLCs as well, especially when free accessible thiol groups were present. The

increased amount of free thiol groups in the gel matrix resulted in significantly higher cell viability and facilitated spontaneous osteogenic differentiation of PDLCs.

We have prepared a novel drug delivery system. Oppositely charged poly(a-amino acid)-based polyelectrolytes interact electrostatically forming polymer nanocomplexes with a diameter 100-200 nm. The size and the shape of the nanocomplexes were characterized by DLS and different microscopic techniques. The pH and salt concentration dependent stability and membrane permeability were investigated as well.

Unidirectional strain-controlled force measurements have been performed on brittle, randomly oriented fibrous scaffold, prepared by electrospinning from polysuccinimide (PSI) based polymers. We observed that the mechanical behavior of these weak 2D networks deviated significantly from that of traditional materials treated usually within the framework of continuum mechanics. These loading curves show a maximum type dependence due to damage formation, that results in stiffness reduction at elongation. We are able to monitor the rupture formation by sensitive force measurements and study how evolution of the ruptures develops during elongation. On the basis of the Fiber Bundle Model, the loading force can be directly related to the cumulative probability distribution function of failures, appearing on the loading curve as abrupt force drops during extension. We are able to determine the sequence of rupture force during extension as well as the magnitude of force drops. Large number of failures observed on each loading curve, made possible to analyze the statistical properties of damage formation.

A new method has been developed for the procedure of highly porous alumina, silica and aluminosilicate cryogels. The methods based on sol-gel and freeze drying techniques. The sol-gel derived wet gels with homogeneous 3D network are provided as a precursor system of the freeze drying. These materials are suitable for preparing a composite system with addition of a biopolymer. Regarding the biomedical application the porous aluminium oxide or aluminosilicate cryogels have been changed to porous silicon dioxide cryogel in the composite systems with biopolymers. On the surface of SiO_2 cryogel many OH groups can be found, which are capable to connect to the organic compounds, such as drugs or biopolymers.

The preparation conditions of silicon dioxide cryogel were modified by catalysts, their concentrations, and additional compounds increasing the porosity. The additives were citric acid, polyethylene-oxide and Pluronic triblock copolymer. The materials were catalyzed and treated by nitric acid, nitric acid + polyethylene-oxide (PO), and nitric acid + Pluronic. According to the results of SEM and FTIR TEOS, ethyl alcohol, and the diluted nitric acid prove to be most effective preparation conditions. In this case the pore size is 1-2 μm and porosity is about 67 %.The composites/ hybrids were prepared in five different ways. At first fibrous systems were fabricated by electrospinning or centrifugal force. On the other hand some composites / hybrids were synthesized from silica hydrogels / alcogels and fibrous polysuccinimide which were dried under lyophilisation process. It was also tried to add some silica cryogel to precursor materials of PSI whereby other systems were obtained. At last it was considered significant to grow the interactions between silica cryogel and PSI phases so a model system was also prepared. This material was synthesized by suspension at the same proportion of silica cryogel and polysuccinimide in DMF, which was held on 60 °C at 24 h. Two synthesis routes were found to be promising. The electrospun fibers were drawn from a suspension of 18-18 m/m % silica cryogel and polysuccinimide; the composite systems were obtained from freeze-dried silica alcogel and dried, fibrous polysuccinimide Especially the electrospun fibrous composite / hybrid having a continuous 3D structure can be applied for artificial tissues.

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