LABORATORY OF TISSUE ENGINEERING

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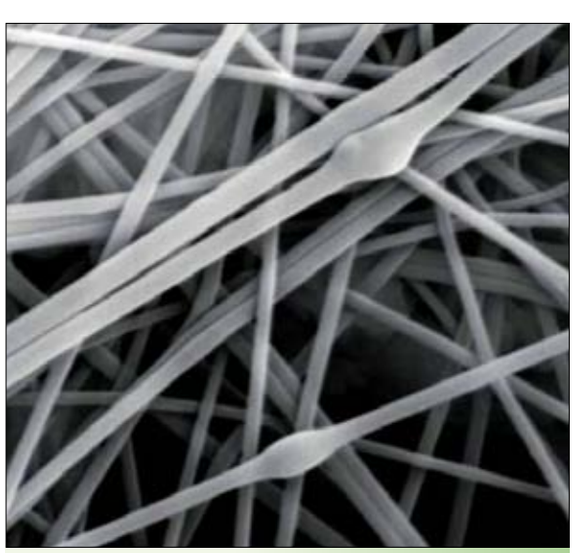
The Laboratory of Tissue Engineering was established in the year 2005 after the research team moved from the Institute of Physiology of the AS CR. In conjunction with the relocation, the main research effort was focused on tissue engineering. Currently, three main research topics are investigated in the laboratory: tissue engineering, controlled drug delivery and protein engineering. The laboratory closely collaborates with the Department of Biophysics, Charles University in Prague, the 2nd Faculty of Medicine and the Department of Nonwovens, Faculty of Textile Engineering, Technical University of Liberec.

RESEARCH TOPICS

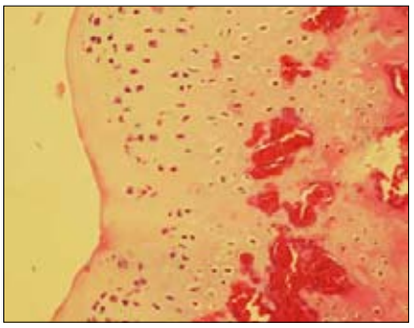
The research is concentrated on the development of novel three-dimensional scaffolds utilizing biodegradable materials. Textiles, both woven and nonwoven, as well as composite scaffolds are generated mainly employing a nanofiber-based approach and

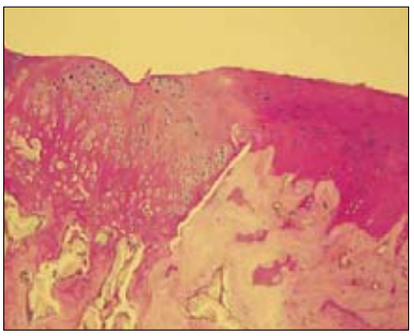


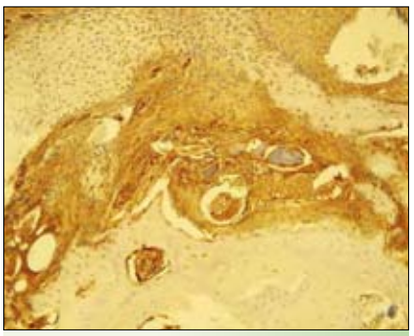
Controlled drug delivery.
Liposome-enriched nanofibers.

applied separately or in combination with an isotropic gel. Grafts based on autologous chondrocytes and mesenchymal stem cells are used for tissue defect regeneration (namely cartilage and bone). A special technique for the rapid evaluation of biomechanical properties in miniature tissue pieces was developed.

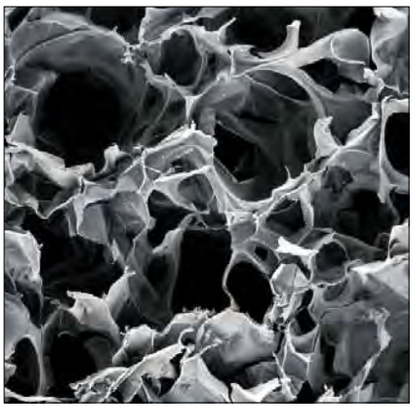
A liposome-based controlled drug delivery and nutrient supply system to deliver bioactive substances directly into defects was developed. The application of the novel technology of coaxial spinning for the production of smart nanofibers is intensively studied, especially in combination with liposomes, with the aim of developing suitable systems for controlled drug delivery. This advanced drug delivery system is mediated with liposome- and immunoliposome-enriched nanofibers and controlled by ultrasound sonication and shock-waves.

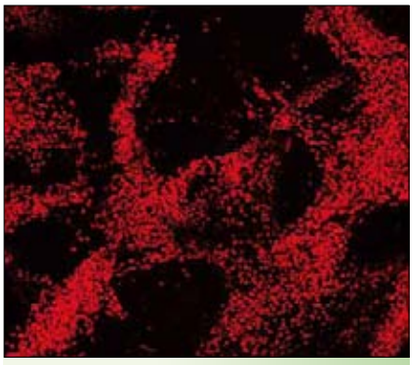






Histology of an osteochondral defect 6 weeks after implantation demonstrates the capability of a composite hyaluronan/type I collagen/fibrin scaffold to regenerate rabbit-knee cartilage.
Hematoxylin–eosin staining. Glycosaminoglycan detection by Alcian blue staining and PAS reaction. Immunohistochemical detection of type II collagen.

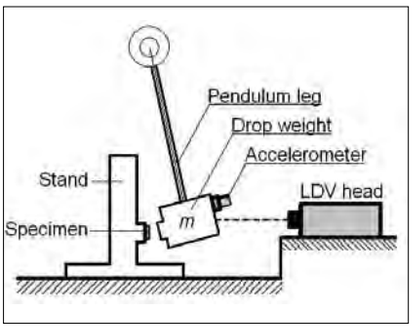


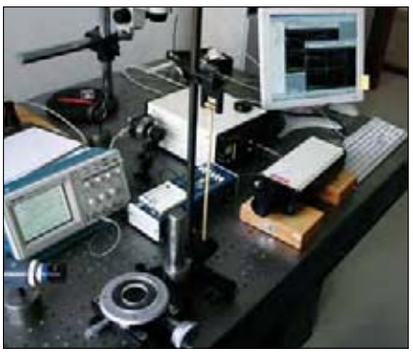


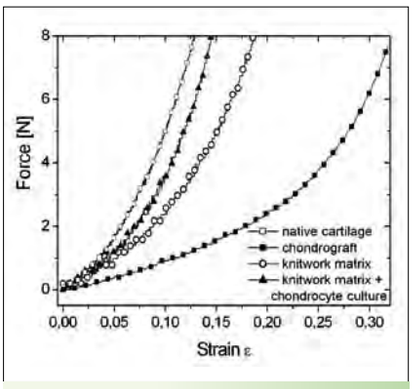
Scaffolds and cell seeding.
Electron microscopy of a cross-linked gelatin scaffold and confocal microscopy of a chondrocyte-seeded scaffold.

Artificial tissue implantation is another research topic. A novel approach was found to improve chondrocyte proliferation, nutrition and re-differentiation capacity, at the same time providing appropriate mechanical stability. The constructed scaffolds seeded with autologous chondrocytes can successfully heal osteochondral defects in experimental animals (rabbits and miniature pigs). Pre-clinical studies, following Good Laboratory Practice, are currently in progress.

The modern approach of computer modeling is applied for predicting the structural properties of cells and tissues, including protein dynamics.



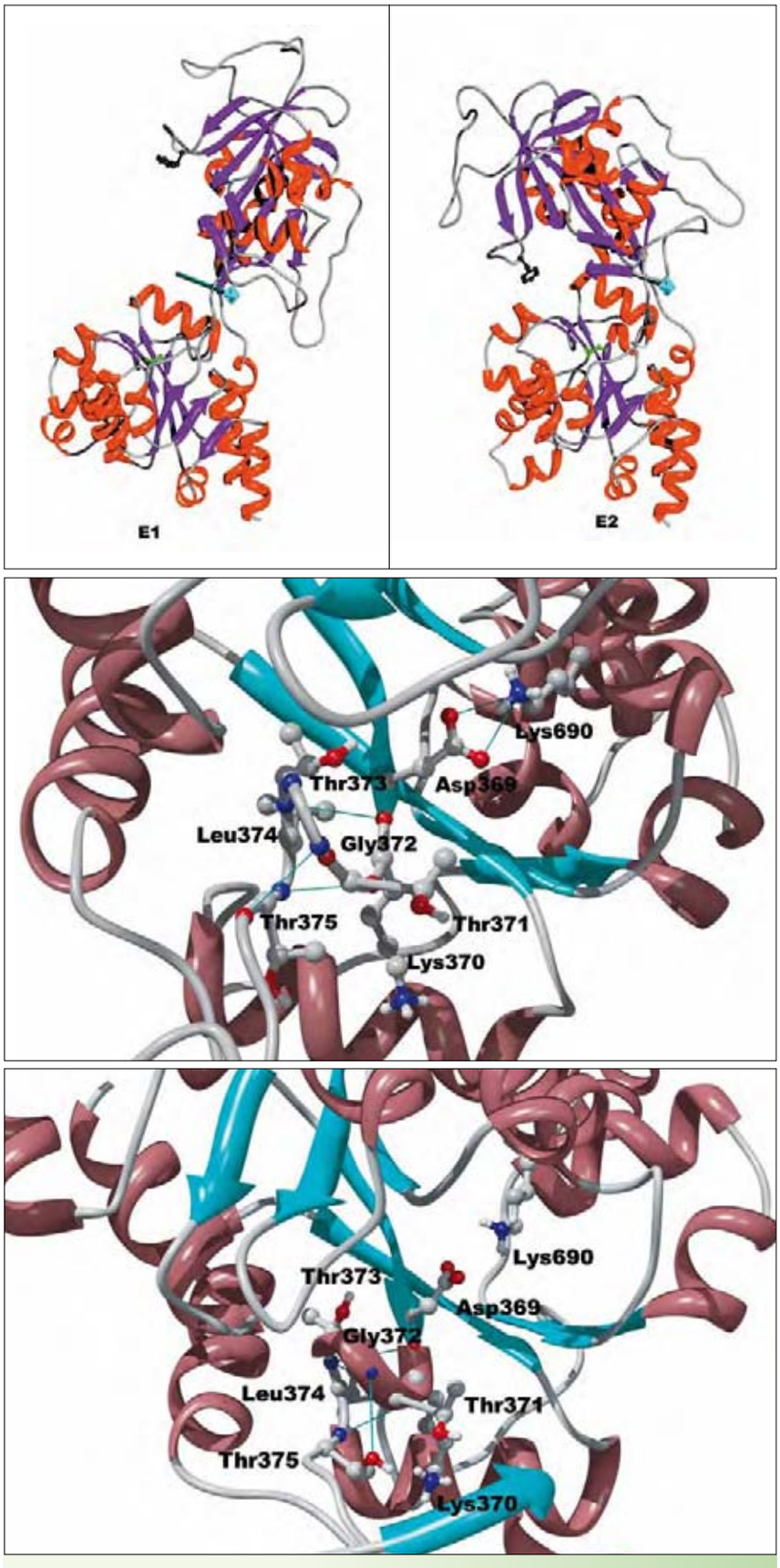




Biomechanical testing.
Scheme and apparatus for impact loading measurement. Loading curves of native cartilage and some of the materials tested.

Computer modeling, based on homology and similarity with proteins of known structure, is focused on protein structure determination and molecular dynamics simulation. Advanced studies of the molecular mechanism of Na^+/K^+-ATPase phosphorylation and of the structure and dynamics of the ATP-binding site on Na^+/K^+-ATPase are carried out. The relation between Na^+/K^+-ATPase structure, function and diseases (specifically familial hemiplegic migraine – FHM2) is also investigated.

High on our priority list is also the accelerated transfer of newly developed technologies and know-how into clinical practice.



Structure of Na^+/K^+-ATPase from mouse brain (α_2 isoform) in both E_1 and E_2 conformations.

Structure of the H_4-H_5 loop of Na^+/K^+-ATPase. The N-domain bends toward the P-domain by 64.8°. Detailed structure of the phosphorylation site in the E_1 and E_2 conformations. A hydrogen bond between the O atom of Asp^{369} and the N atom of Lys^{690} detected in the E_1 conformation disappears in the E_2 conformation, accompanied by the appearance of the short π-helix.

CURRENT GRANT SUPPORT

GA AS CR, IAA500390702, Scaffolds from liposome nanofibers for tissue engineering, 2007–2011.

Ministry of Education, NPV II 2B06130, Synthesis of new biomaterials and preparation of stem cell derived cells, and their applications in for the treatment of diseases affecting human tissues derived from mesoderm: cartilage, bone, ligament and meniscus, 2006–2011.

EU 7th FP, ID number 214539, Bioscent – Bioactive highly porous and injectable scaffolds controlling stem cells recruitment, proliferation and differenciation and enabling angiogenesis for cardiovascular engineered tissues, 2009–2013.

GA AS CR, Institutional research plan AV0Z50390512, Molecular, cellular and systemic mechanisms of major diseases of the human organism, their diagnosis, therapy and pharmacotherapy, 2005–2011.

GA AS CR, Institutional research plan AV0Z50390703, New biotechnologies, nanomaterials and stem cells for use in regenerative medicine, 2007–2013.

PATENT

Patent No. 300142 – The way of preparation of fibrous biodegradable tectorial materials as drug carriers with controlled delivery. Industrial Property Office (Czech Patent and Trademark Office), 25. 2. 2009, Filing Date: 22. 3. 2006.

SELECTED RECENT PUBLICATIONS

1. Filová E, Rampichová M, Handl M, Lytvynets A, Halouzka R, Usvald D, Hlučilová J, Procházka R, Dezortová M, Rolencová E, Košťáková E, Trč T, Šťastný E, Koláčná L, Hájek M, Motlík J, Amler E. (2007) Composite hyaluronate-type I collagen-fibrin scaffold in the therapy of osteochondral defects in miniature pigs. Physiol Res 56 Suppl. 1., S5–S16.

2. Koláčná L, Bakešová J, Varga F, Košťáková E, Plánka L, Nečas A, Lukáš D, Amler E, Pelouch V. (2007) Biochemical and biophysical aspects of collagen nanostructure in the extracellular matrix. Physiol Res 56 Suppl. 1., S51–S60.

3. Varga F, Držík M, Handl M, Chlpík J, Kos P, Filová E, Rampichová M, Nečas A, Trč T, Amler E. (2007) Biomechanical characterization of cartilages by a novel approach of blunt impact testing. Physiol Res 56 Supl. 1., S61–S68.

4. Míčková A, Tománková K, Kolářová H, Bajgar R, Kolář P, Šunka P, Plencner M, Jakubová R, Beneš J, Koláčná L, Plánka L, Nečas A, Amler E. (2008). Ultrasonic shock-wave as a control mechanism for liposome drug delivery system for possible use in scaffold implanted to animals with iatrogenic articular cartilage defects. Acta Vet Brno 77: 285–289.

5. Tejral G, Koláčná L, Schoner W, Amler E. (2008). The π-helix formation between Asp^{369} and Thr^{375} as a key factor in E^1-E^2 conformational change of Na^+/K^+-ATPase. Physiol Res *in press*

6. Filová E, Jelínek F, Handl M, Lytvynets A, Rampichová M, Varga F, Činátl J, Soukup T, Trč T, Amler E. (2008) Novel composite hyaluronan/type I collagen/fibrin scaffold enhances repair of osteochondral defect in rabbit knee. J Biomed Mater Res B Appl Biomater 87B(2): 415–424.