

# Department of Growth and Differentiation of Cell Populations



is a member of the Institute of Physiology  
& Centre for Cardiovascular Research,  
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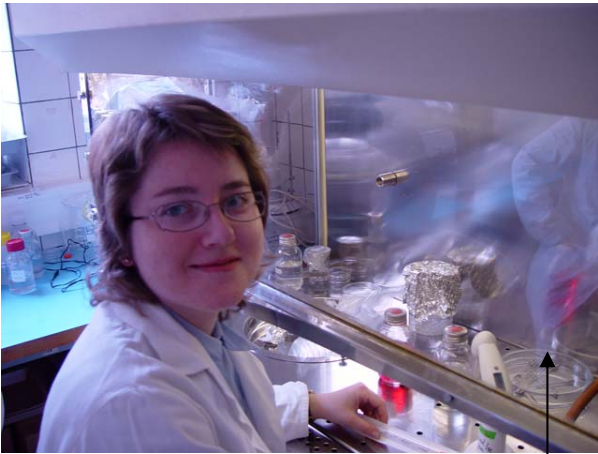
Martin Parizek, M.Sc.

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# Laboratory life



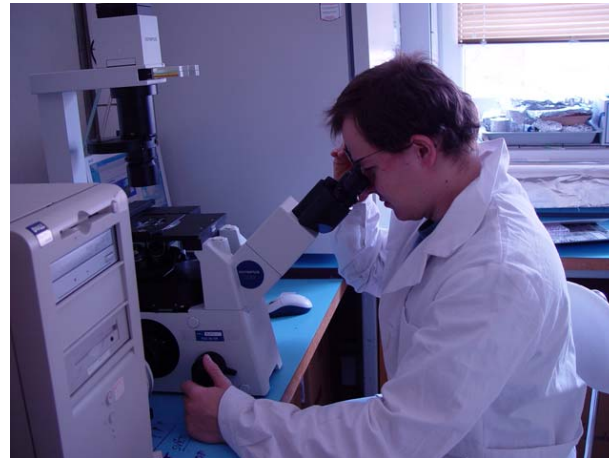
**Tissue culture room**



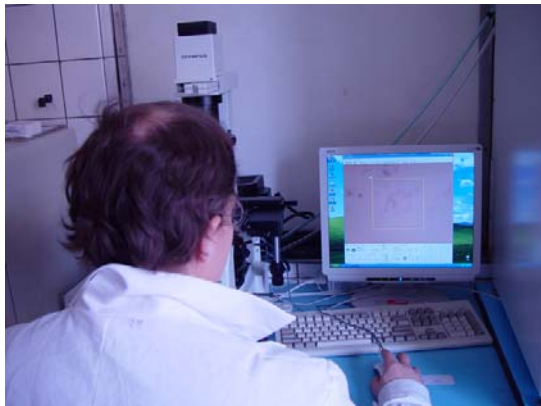
**Immunocytochemistry,  
histology & biochemistry lab**



**& inverted Olympus  
fluorescence microscope  
with digital camera**



**and image analysis**

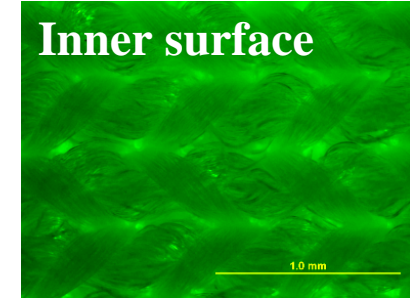
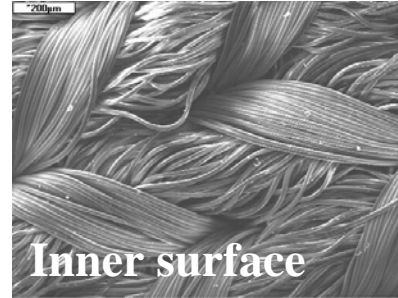


# Tissue engineering program

1. Innovation of clinically used vascular prostheses by their endothelialization
2. Construction of bioartificial vascular tissue
3. Regionally-selective cell adhesion on micropatterned surfaces
4. Bone-derived cells on nanostructured materials
5. Bone tissue engineering in three-dimensional scaffolds



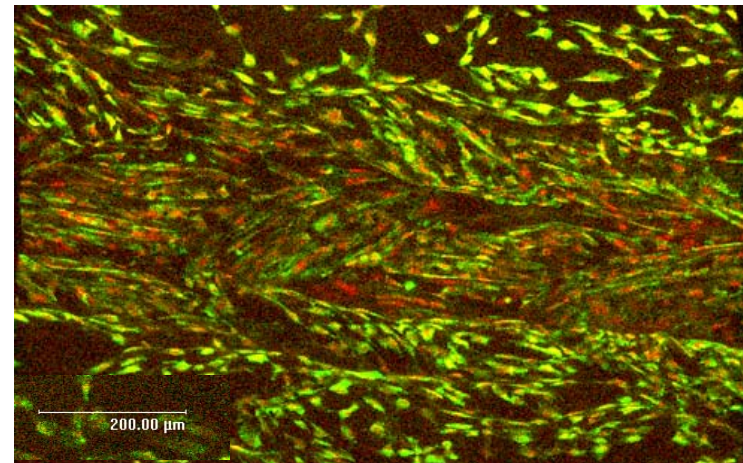
# 1. Innovation of clinically used vascular prostheses: coating with entire extracellular matrix (ECM) molecules and endothelialization



Knitted crimped polyethylene terephthalate (PET) vascular prosthesis, 6 mm ID, impregnated with collagen I, produced in the VÚP® Joint-Stock Comp., Brno, Czech Rep.

On the inner surface, **laminin** or **fibrin** were immobilized

The inner surface was then seeded with **human saphenous vein endothelial cells** (HSVEC) obtained at coronary bypass surgery ( $1.5 \times 10^5$  cells /  $\text{cm}^2$ )  
On day 2 after seeding, **the cells were exposed to 120 min laminar shear stress (15 dynes/ $\text{cm}^2$ ) in a perfusion system** (in cooperation with Inserm U577, Université V. Segalen, Bordeaux, France; Prof. Laurence Bordenave)

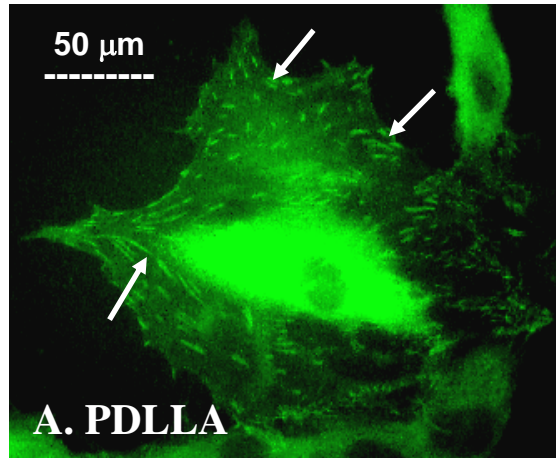


Confocal  
Microscope  
Leica TCS  
SP2 AOBS  
39 x 10  $\mu\text{m}$ ,  
obj. 10x

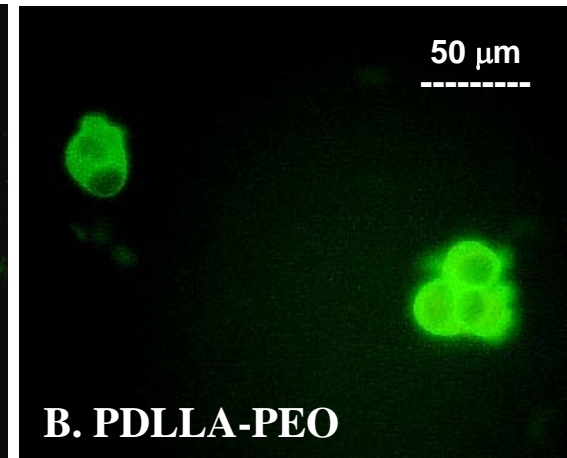
Immunofluorescence staining of **von Willebrand factor**, a marker of endothelial cell identity and differentiation, in HSVEC. The cell nuclei were counterstained with **propidium iodide**.

## 2. Construction of bioartificial vascular tissue: vascular smooth muscle cells on polymers with ECM-derived ligands for cell adhesion receptors

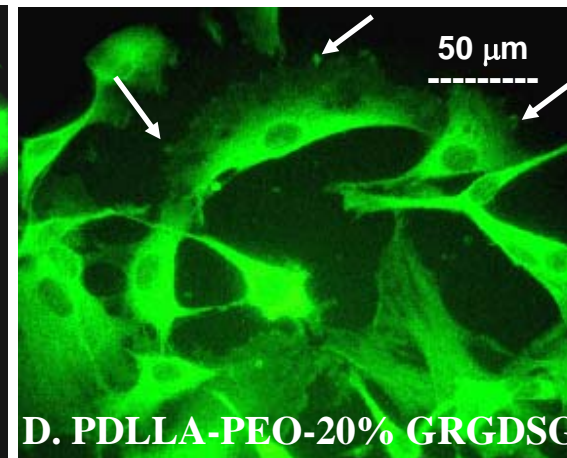
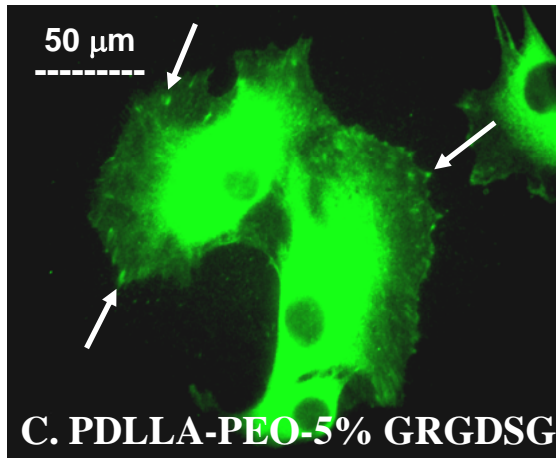
A. **Poly D,L lactide (PDLLA)**: cell adhesion through proteins adsorbed from the culture medium (vitronectin, fibronectin). Cells are well spread and form vinculin-containing focal adhesion plaques (arrows).



B. **Copolymer of PDLLA and polyethylene oxide (PEO)**: extremely hydrophilic PEO prevents uncontrolled protein adsorption and cell adhesion.



C, D: **Functionalization of 5% or 20% of PEO chains with the oligopeptide GRGDSG**, a ligand for integrin adhesion receptors, restored (at least partly) the cell adhesion, spreading and assembly of focal adhesion plaques (arrows).



Imunofluorescence staining of vinculin, a protein of focal adhesion plaques, day 3 after seeding. **Polymeric materials prepared in the Institute of Macromolecular Chemistry, Acad. Sci. CR**

### 3. Regionally-selective cell adhesion on micropatterned surfaces

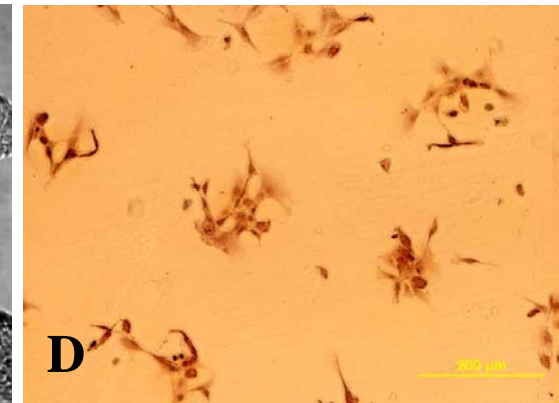
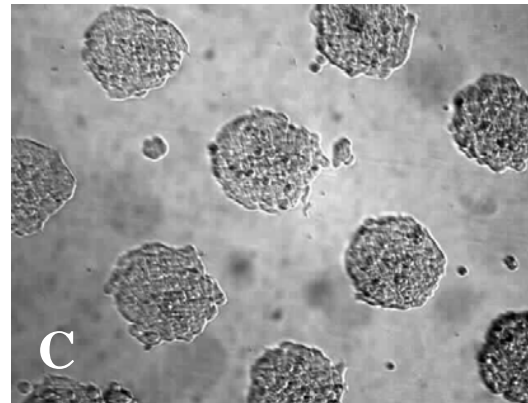
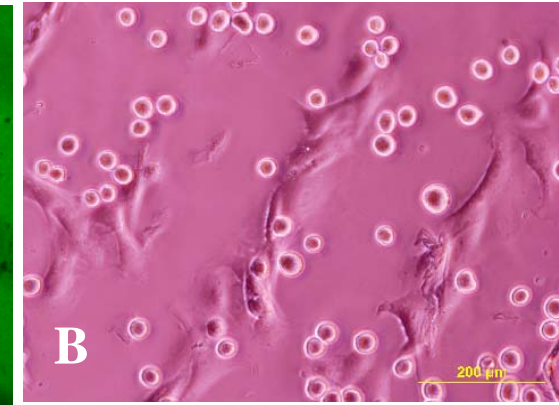
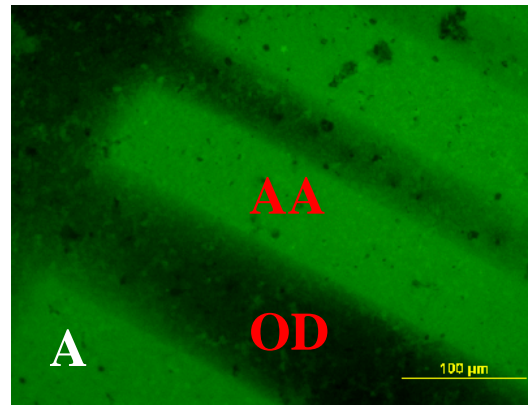
**A, B.** Microstructured surfaces created by plasma polymerisation of hydrophilic acrylic acid (AA) and hydrophobic 1,7- octadiene (OD).

**A.** Preferential adsorption of fluorescence-labelled collagen IV on AA domains.

**B.** Preferential adhesion of vascular smooth muscle cells on AA domains.

**C.** Preferential adhesion of vascular endothelial cells on microdomains created by UV-light irradiation of polytetrafluoroethylene through a metallic mask in  $\text{NH}_3$  atmosphere.

**D.** Preferential adhesion of vascular smooth muscle cells on microdomains created by the irradiation of polyethylene with  $\text{Ar}^+$  ions (energy 150 keV, dose  $10^{13}$  ions/cm<sup>2</sup>).



Micropatterned surfaces are useful for obtaining regionally-selective cell adhesion on materials for tissue engineering or in microarray technique for advanced research in genomics and proteomics.

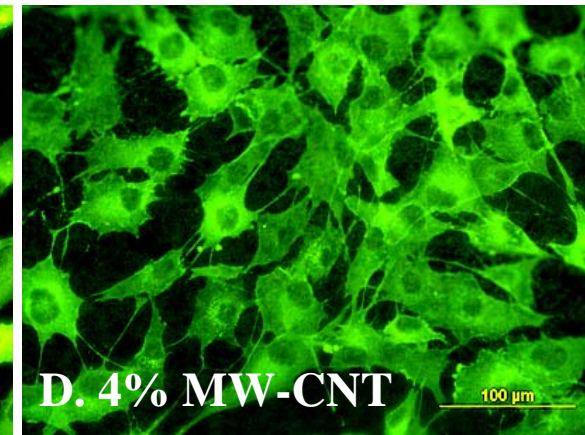
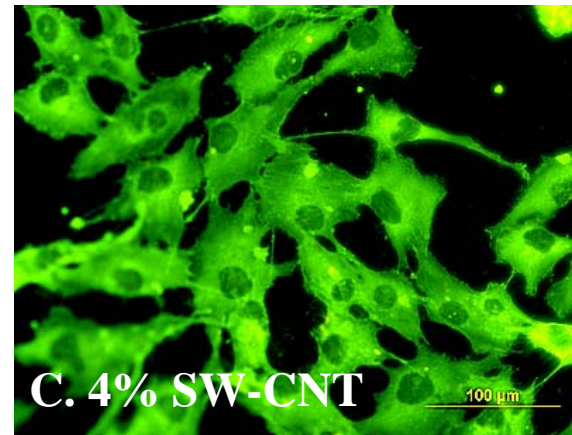
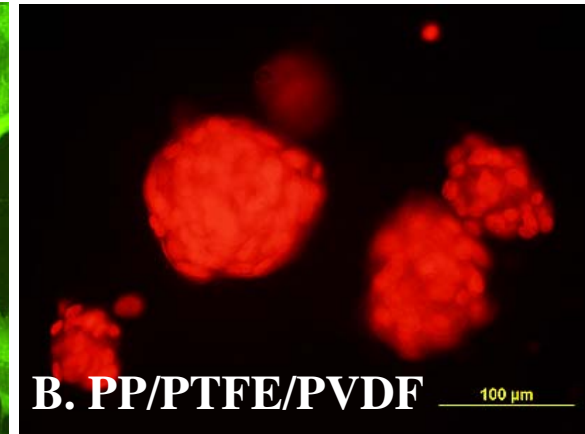
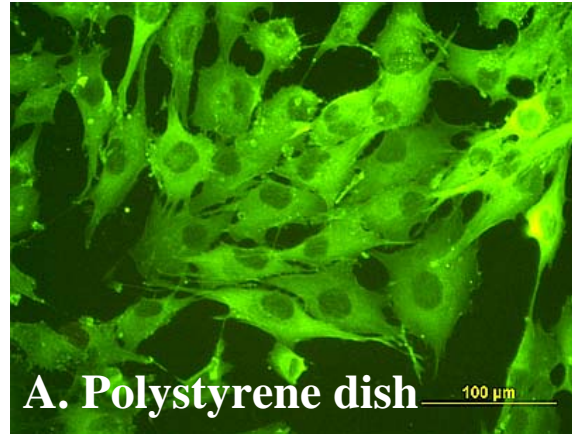
Materials were prepared in the cooperation with the Department of Engineering Materials, University of Sheffield, Sheffield, UK (A, B), Angewandte Physik, Johannes Kepler Universität, Linz, Austria (C) and the Institute of Chemical Technology, Prague, Czech Rep. (D)



## 4. Human osteoblast-like MG 63 cells on nanostructured materials

- A. Well spread MG 63 cells on standard tissue culture polystyrene.
- B. Round, non-spread and aggregated cells on highly hydrophobic terpolymer of polypropylene, polytetrafluorethylene and polyvinylidene difluoride.
- C, D. The cell adhesion and spreading was restored when the nanostructure of the material surface was created by mixing single- or multi-walled carbon nanotubes with the terpolymer.

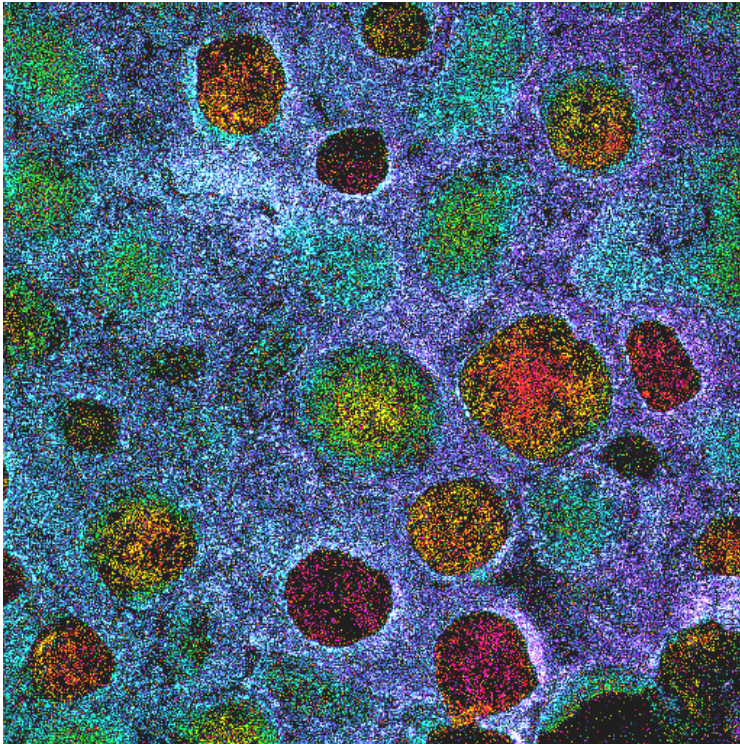
Materials were prepared in cooperation with the AGH University of Science and Technology, Krakow, Poland



**Immunofluorescence of beta-actin cytoskeleton (A, C, D) or propidium iodide staining of cell nuclei and partially cell cytoplasm (B), day 3 after seeding, microscope Olympus IX 50, digital camera DP 70, obj. 20x**



## 5. Human osteoblast-like MG 63 cells in “three-dimensional” porous scaffolds for bone tissue engineering



Cells in depth of:

**0 – 60  $\mu\text{m}$  (blue signal)**

**80 – 160  $\mu\text{m}$  (green)**

**180 – 220  $\mu\text{m}$  (yellow)**

**240 – 300  $\mu\text{m}$  (orange)**

**320 – 400  $\mu\text{m}$  (red)**

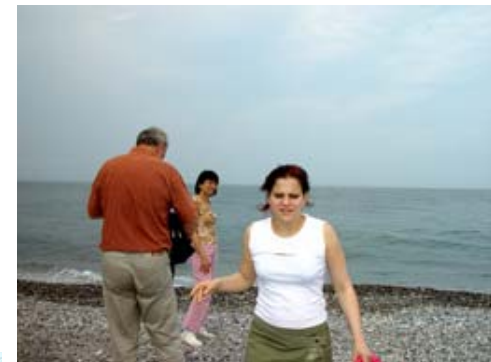
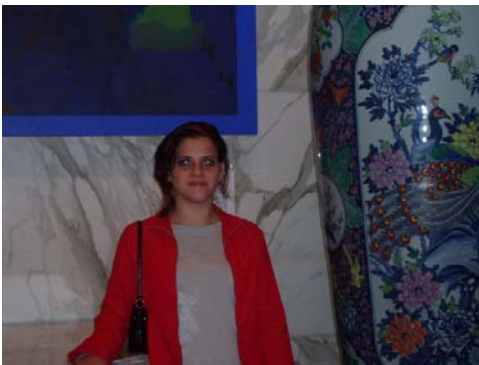
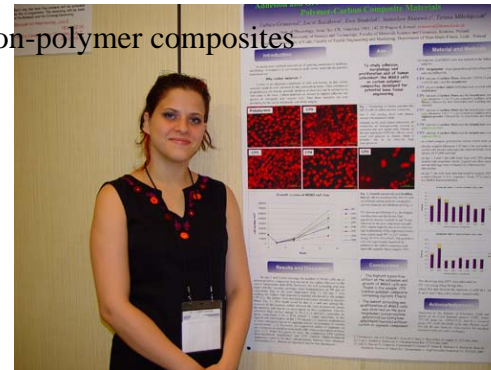
**420 – 480  $\mu\text{m}$  (violet)**

- ❖ Scaffolds were made of a copolymer of lactide and glycolide
- ❖ Collaboration with the AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Krakow, Poland
- ❖ Average pore size and depth was about 400  $\mu\text{m}$
- ❖ Seeded with  $\sim 70\,000$  cells/ $\text{cm}^2$ , cultured in the medium DMEM with 10% of fetal bovine serum for 14 days
- ❖ Cells fixed with ethanol, stained with propidium iodide
- ❖ Examined in confocal microscope (Leica TCS SP2, Germany) using transversal optical sections through pores every 20  $\mu\text{m}$



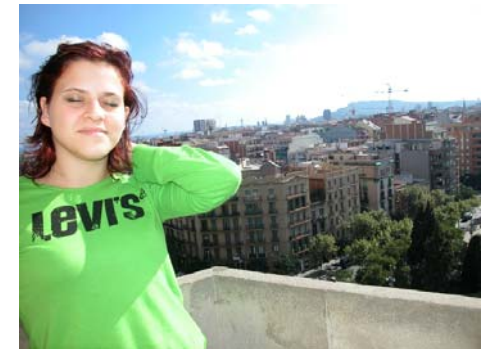
# Carbon 2005

International Conference on Carbon, July 3-7, Gyeongju, Korea





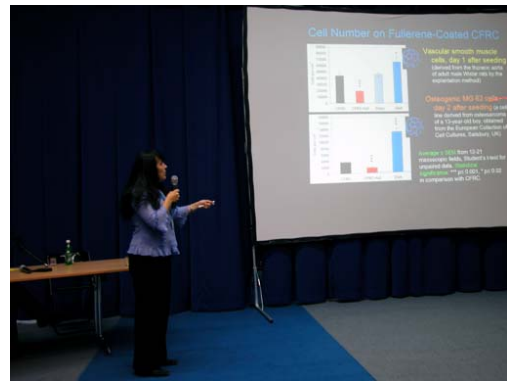
# Plasma Polymers and Related Materials, Joint Meeting of the COST, Workgroup 527, Sant Feliu de Guixols near Barcelona, Spain; October 2005





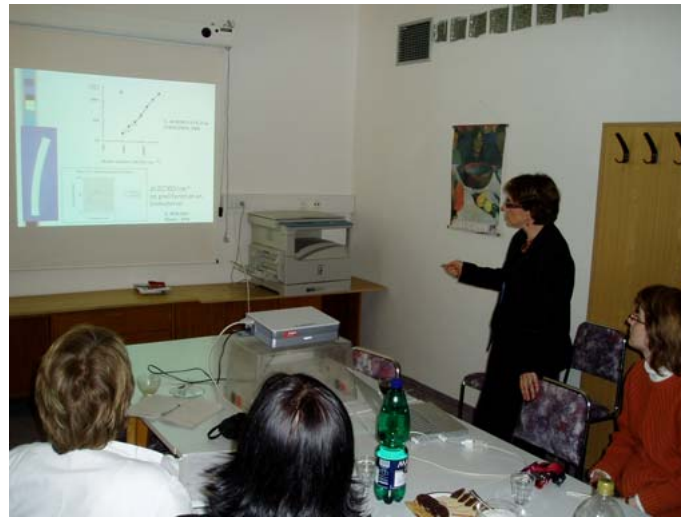
# XV Conference on Biomaterials in Medicine and Veterinary Medicine

Rytko, Poland, October 13-16, 2005



# Visit of French scientists in our lab, November 2005

Université Victor Segalen, l'Unité INSERM 577, Laboratoire de Biophysique, Bordeaux, France; collaboration within **Barrande** project



**Prof. Laurence Bordenave** giving a lecture on vascular biology & tissue engineering



Chantal Bourget

Murielle Rémy-Zolghadri

Prof. L. Bordenave



**Confocal microscopy of bioartificial vascular prostheses**

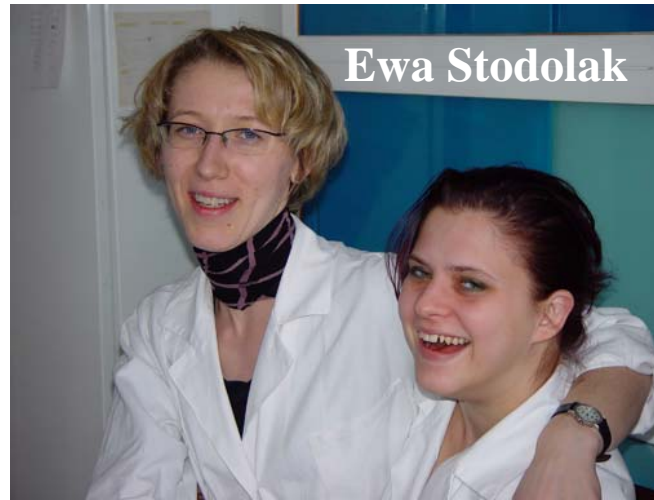


# Visit of Polish scientists in our lab, February 2006

AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Dept. of Biomaterials, Krakow, Poland



**Dr. Elzbieta Pamula**



**Ewa Stodolak**

Collaboration on bone tissue engineering (bone cells on polymeric and carbon materials, including those three-dimensional and nanostructured)





# Join our vascular and bone tissue engineering team!

