

In 2003 Dr. Miep Helfrich received an ECTS exchange grant to visit the Laboratory of ECTS member Prof. Jenneke Klein-Nulend at the Free University in Amsterdam to study the response of osteoblasts to fluid shear stress. She was particularly interested to visualise the enzyme endothelial nitric oxide synthase (eNOS) in osteoblasts before and soon after exposure to fluid shear to test the hypothesis that re-location of the enzyme from caveolae to the cytoplasm may be required for activation of the enzyme and the rapid NO production seen in bone cells exposed to fluid shear. To study the subcellular localisation of eNOS she wanted to use immuno-electron microscopical analysis and she requested help with this technique from the group of Judith Klumperman, at the Academic Medical Center in Utrecht, The Netherlands. In Amsterdam, a time series of flow experiments was carried out and cells were fixed in fixatives recommended by the Utrecht group who had experience with eNOS immunolocalisation in human cells. Fixed cell pellets were immersed in sucrose for freezing followed by ultrathin cryosectioning. Training in cryosectioning and immunogold labelling of sections was obtained in Utrecht. Unfortunately it transpired that the recommended antibody to detect eNOS is not specific for this enzyme in mouse cells and no conclusive data were obtained. However, the exchange grant gave the applicant a great opportunity to learn a new technique that had not previously been applied to bone cells (cryo-immuno-EM) and strengthened the links between the Aberdeen group and the Amsterdam group. In addition, the cell preparation and sectioning technique has now been successfully introduced in the lab in Aberdeen and further studies on eNOS activation are ongoing.