Internship Vacancy: The effect of chronic hyperglycemia induced by diabetes on bone mineralization, 6 months at Biochemistry, Radboudumc

Title of the project	**The effect of chronic hyperglycemia induced by diabetes on bone mineralization**
Contact person	Elena Macías Sánchez
	Elena.macias@radboudumc.nl
Daily supervisor	Luco Rutten
Email-address	Luco.rutten@radboudumc.nl
	Group: Biochemistry of Mineralized Tissues group, Department: Biochemistry, Radboudumc
Interested mail:	Luco.rutten@radboudumc.nl & Elena.macias@radboudumc.nl
Topic	Nanomedicine
Techniques	Cell culture, high performance liquid chromatography (HPLC), cryo-TEM
Time period (months)	6 months
Short description of the proposed internship	Diabetes mellitus is a disease which is characterized by a high sugar concentration over longer times. Chronic hyperglycemia results in a complex series of reactions between glucose and amino acid residues (glycation) of long-lived proteins. This reaction forms advanced glycation end-products (AGEs). One of these long-lived proteins is collagen, which is the main organic component in bone. Collagen molecules assemble into fibrils and are subsequently mineralized with hydroxyapatite. The mineralized collagen fibril is the basic building block of the bones in our body. A well-known complication of diabetes is a decrease in the quality of the bones, which results in higher fracture risk. We hypothesize that newly formed crosslinks induced by AGEs, result in changes in the micro-structure of the fibril causing impaired mineralization of the fibril. The goal of this project is to investigate the influence of glycation on the mineralization of collagen. To validate this hypothesis you will, in the first couple months, produce collagen fibrils with an increased level of glycation. The increase of glycation should be comparable to the known degree of glycation from patients suffering from diabetes. This could be achieved by incubating isolated collagen produced by an osteoblast cell culture in a solution containing high concentrations sugars. In parallel you will set-up a method to quantify the amount of AGEs on the collagen. High performance liquid chromatography (HPLC) is ideal to determine the degree of the most common AGEs on collagen. The collagen will be hydrolyzed and the amino acids are separated on the HPLC column. The amount of AGEs will be quantified by measuring the fluorescent intensity of the derivatized the amino acids. Once the AGEs have been quantified and the collagen isolated from the cell culture, you will use this collagen to evaluate the mineralization. An osteogenic mineralization solution can be used to mineralize the isolated collagen fibril *in-vitro*. The mineralization will be inspected using electron microscopy. You will apply cryogenic Transmission Electron Microscopy (cryo-TEM), a technique that allows the observation of proteins in near-native conditions by ultra-rapid freezing of the samples (vitrification). You will use an automated robot (Vitrobot) to capture the sample in a thin layer of amorphous ice, arresting the mineralization process at different timepoints. Subsequently, the sample will be imaged in cryo-conditions and the mineralization time, size and orientation of the crystallites, and amount of mineralization will be assessed.