

Marie Curie European Reintegration Grants (ERG)

Call: FP7-PEOPLE-2007-2-2-ERG

Project title: Bioactive resorbable scaffolds for drug delivery in bone tissue engineering

Project acronym: BIORESS

Period covered: 15.01.2008 – 14.01.2011

Bone replacements are frequently required to substitute damaged tissue due to any trauma, disease or surgery. The current therapies for bone grafts: autografts (taken directly from the patient), allografts (taken from a cadaver donor) or xenograft (taken from animals), are limited in availability and can be associated with patient morbidity or with the risk of inducing transmissible diseases. Due to the numerous restrictions that biologic grafts present, the development of synthetic materials and their employment in tissue engineering implants is continuously increased. The development of synthetic bone substitutes as drug delivery systems has been one of the most important topics in the bone tissue engineering field. A valid solution is the realisation of synthetic tissue-engineered bone scaffold. A bone scaffold is a three-dimensional (3D) porous structure that can be used as a bone graft or bone filling material. It should have good mechanical properties to support the structural loads and high porosity to allow tissue ingrowth and vascularisation, cell adhesion, migration and proliferation. Scaffolds made of phosphate glasses are an attractive solution. Phosphate glasses are resorbable and the degradation kinetics can be controlled by their composition. By choosing appropriate chemical composition, the scaffold dissolution rate can be tailored similar to the one of the bone *in vivo*. Due to their glass characteristics, their surface can be easily modified, in order to improve their integration into natural tissue. By a suitable functionalisation process, the surface becomes biologically active and proteins adsorption can be enhanced. Protein adsorption is the first stage in cell adhesion and proliferation on the biomaterial surface. Moreover, the modified surface will be successively used to bind specific macromolecules (enzymes, antibiotics, growth factors, growth hormones, chemiotherapeutic agents, anti-inflammatory drugs etc.) for local drug delivery. Therefore, functionalised resorbable scaffolds combine the excellent biocompatibility, bioactivity (the ability to develop a chemical bond with the newly formed bone tissue, by precipitation of hydroxylapatite layer similar with the one found in natural bone) and osteoconductivity (the ability to support bone growth over its surface) with the possibility of adding drugs, making them useful as bone fillers and drug delivery systems.

The aim of this project was the development, characterisation and *in vitro* experimentation of novel macroporous scaffolds with highly interconnected porosity and controlled biodegradability for drug delivery in bone tissue engineering. The **main objectives** of this project were:

1. Synthesis and characterisation of bioactive and bioresorbable phosphate glasses
2. Synthesis and characterisation of bioactive and bioresorbable scaffolds
3. *In vitro* biological characterisation of the scaffolds for drug delivery systems

Phosphate glasses belonging to the complex system $P_2O_5-SiO_2-Na_2O-CaO-MgO-K_2O$ were obtained by a traditional melting and quenching method. They were then characterised in terms of microstructure, solubility and bioactivity. The glass structure was analysed by X-ray diffraction (XRD). The glass solubility was tested at 37°C on glass slices by using three different solutions: distilled water, simulated body fluid (SBF) and Tris-HCl, for a soaking time of 4 months. The weight loss per unit area and the pH variation were registered in function of immersion time. The

glass bioactivity was also evaluated during soaking small glass slices in SBF. The formation of hydroxylapatite (HA) layer on the glass surface was analysed by XRD, scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS). The sintering process of the glass was directly observed by differential scanning calorimetry and hot stage microscopy. The results show that the glass is bioactive (a HA layer is formed on the glass surface after immersion in a SBF) and bioresorbable. It lost almost 76 wt.% after 4 months of immersion in distilled water. The dissolution takes place by a congruent surface erosion mechanism.

The glass was further used to produce macroporous 3D-scaffolds for bone regeneration. The scaffolds were fabricated by using the burning-out method. This method, involves the use of an organic phase as porogen, which is mixed with inorganic particles (the glass powder in this case). The mixture is pressed, obtaining a green body, and then it is heat treated to remove the organic phase (by burning) and to sinter the inorganic particles. The sintering conditions of the scaffolds (temperature and time) were chosen according to the thermal properties of the phosphate glasses (previously studied), in order to avoid the complete glass crystallisation. The heat treatment process was optimised in order to provide a better control of the scaffold microstructure and to enhance the mechanical properties. The obtained scaffolds were characterised by X-ray diffraction, scanning electron microscopy and solubility tests in SBF. The scaffolds porosity and their compression strength were also determined. The pores size distribution was evaluated by using image analysis on different SEM micrographs. Biocompatibility was evaluated in vitro, using human marrow stromal cells.

The results show that the obtained scaffolds have a porosity of 80-90vol%, with a high degree of interconnection. The maximum compressive strength was 2 ± 0.5 MPa. After 4 months of soaking in SBF, the scaffolds lost almost 76 wt.% with a pH not exceeding the 7.45 value. Morphological and biochemical assays with human marrow-derived stromal cells seeded on scaffolds showed that the cells maintain their metabolic activity and proliferate on the scaffolds.

To conclude, during this project, a new bioactive and bioresorbable phosphate glass was produced and characterised. This glass was then utilised to produce bioactive and bioresorbable scaffold with highly porous structure and interconnected porosity for bone tissue engineering. It has shown a stimulatory effect in promoting osteogenesis. Due to its high porosity and interconnectivity, the scaffolds can be used as drugs carriers for drug delivery systems.

Due to the high relevance of the project in the bone tissue engineering field, the researcher Dr. Oana Bretcanu participated at several international conferences, workshops and scientific meetings in the fields of Biomaterials, Glass Technology and Materials Science. Three papers were submitted to Peer Reviewed Journals and two of them were already published in a Peer Reviewed Journal. In 2009, the scaffolds were exhibited in the "Aula Magna" amphitheatre of Politecnico di Torino for the celebration of 100 years of graduation of the first woman engineer at Politecnico, with participation of the oldest living Nobel laureate, Dr. Rita Levi-Montalcini.

This project contributed to the development of a new course for PhD students and different experimental laboratories at Politecnico di Torino. Moreover, the project reinforced and developed new collaboration with other national and international research centres and universities in Europe, extending the existing multidisciplinary research network.