

HUMAN BONE FETAL CELL AND POLYMER BIOCOMPOSITE FOR BONE TISSUE ENGINEERING

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INTRODUCTION

Scaffolds for bone tissue engineering were initially composed of either polymer or ceramic, which however tended to be too flexible or too brittle respectively. In the past few years, polymer/ceramic composites have therefore gained increased interest in the field of tissue engineering^{1,2}, to reconstruct several types of structural tissues, such as bone, cartilage, tendons or ligaments, and tissue interfaces. The composite is expected to have improved mechanical properties compared to the neat polymer, and better structural integrity and flexibility than brittle ceramics. In fact the combination of ceramic and polymer could provide reinforced porous structures with enhanced bioactivity and controlled resorption rates³. In the case of bone, scaffolds should ideally match bone properties, i.e. high and interconnected porosity, oriented pores, compressive anisotropy and viscoelasticity.

The aim of the "Lausanne Center for Bone Tissue Engineering" project is to develop an artificial bone, which consists in human bone fetal cells filling the volume of a porous biodegradable composite scaffold. The human bone fetal cells, due to their immunological induced-tolerance will act as osteoinductive factor and the new polymer reinforced scaffold due to its mechanical properties, will act as osteoconductive factor. By combining human bone fetal cells and a biocomposite polymer, an artificial bone can be assembled in the surgical room providing a flexible cost-effective solution to the bone graft problematic.

BIOMECHANICAL CONSIDERATIONS FOR CLINICAL APPLICATIONS

In order to obtain designed mechanical properties for the scaffold, a numerical model (FEM) of tibial osteotomy was developed⁴ (Figure 1a).

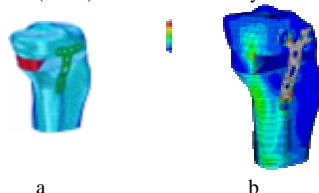


Figure 1: a) View of a reconstructed human tibia in which an osteotomy has been simulated. b) Von Mises Stress distribution over an osteotomized tibia (10°) with a scaffold on the lateral side.

An optimal configuration (Figure 1b) has been found in the lateral scaffold placement with E modulus of 500 MPa, for the following reasons: 1) the maximal bone stress is minimized, 2) the maximal stress in the support is acceptable, and is near to its asymptotic value; 3) the maximal stress in the scaffold material is relatively low.

BIOPOLYMER SELECTION

We tested as a first biocompatibility screening the cytotoxicity of the polymers (PLA, PLGA) and ceramics (HA, β -TCP)⁵. Primary human bone cells were seeded in the presence of PLA, PLGA, HA, β -TCP on 6 cm Petri dishes at density of 10^5 cells/dish. Results showed that bone cells were able to proliferate in all groups. For example, the polymer PLGA was totally surrounded by cells that seem to anchor to it, while in the presence of ceramics, the cells proliferation was not disturbed (Figure 2).

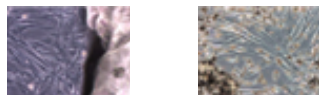


Figure 2: Proliferation of primary human bone cells around the PLGA polymer (left) or in the presence of β -TCP particles (right).

BIOCOMPOSITE SCAFFOLD DEVELOPMENT

Based on the biomechanical considerations for clinical applications and the general requirements for a bone scaffold, a biocomposite polymer was developed^{6,7}. A commercial bioresorbable polymer, poly L-lactic acid (PLA, Boehringer Ingelheim, Germany) was used. Two ceramic powders were added to PLA: hydroxyapatite (HA) (Merck KGaA,

Darmstadt, Germany) and β -tricalcium phosphate (β -TCP) (Fluka, Buchs SG, Switzerland). After a mixing of particles and polymer by melt extrusion process, the composite was foamed by supercritical CO_2 technique. Pressure was increased up to saturation pressure P_{sat} (150-250 bar), and temperature increased up to 195°C , above the PLA melting point. Polymer saturation by CO_2 was completed after 10 min. Foaming was then achieved by sudden gas release, with simultaneous gas cooling and consequently foam cooling also. Initial depressurization rate dP/dt and maximum cooling rate dT/dt are significant parameters which affect pore expansion and stabilization. Different processing conditions and ceramic contents resulted in various porous structures (Figure 3):



Figure 3: The effect of foaming parameters on PLA/ceramic foam morphology (3D μ -CT reconstructions).

Neat and composite polymer foams exhibited anisotropy in morphology with pores oriented along the foaming direction. Compression tests were carried out in order to evaluate if they also presented anisotropy in their mechanical behaviour, induced by this anisotropic macrostructure. The results of compression experiments are presented in Figure 4

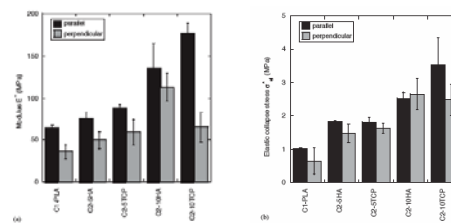


Figure 4: The effect of testing direction on compressive properties of neat and composite polymer foams. a) Modulus E^* ; and b) Elastic collapse stress σ_{el}^* (for example, C2-5HA means PLA with 5% HA).

SCAFFOLDS IN VITRO AND IN VIVO EVALUATIONS

When seeded directly on the scaffolds, fetal and adult bone cells did proliferate and differentiate in their immediate vicinity⁸. Furthermore, both adult and fetal cells were able to spread on scaffold surfaces, independently of the foaming condition and of the presence or not of ceramic fillers. In parallel, cell differentiation toward osteoblasts was demonstrated by alkaline phosphatase (ALP) enzymatic activity, γ -carboxylated Gla-osteocalcin production (Gla-OC), and the onset of mineralization. The addition of HA or β -TCP resulted in a higher ALP enzymatic activity for fetal bone cells and a stronger production of Gla-OC for adult bone cells.

In vivo evaluation of the biocomposite scaffolds is currently performed in a Sprague-Dawley rat critical size defect (CSD) craniotomy model. The preliminary results show a mild inflammation reaction at 12 days and 4 months. Bone ingrowth in the scaffold was also observed at 4 months.

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