Characterization of cell cultures in contact with different orthopedic implants biomaterials

G Ouenzerfi1,3, A Hannoun1, M Hassler3, I. Brizuela2, S Youjl2, C Bougault2 and A-M Trunfio-Sfarghiu1

1 Contact and structure mechanics laboratory - INSA of Lyon
2 Institut de Chimie et de Biochimie Moléculaires et Supramoléculaires (ICBMS) ;ODMB laboratory
3 Wright Medical

E-mail: ghassene.ouenzerfi@insa-lyon.fr

Abstract. The aim of this study is to identify the role of biological and mechanical constraints (at the cellular level) surrounding living tissues (cartilage and bone) in the presence of different joint implant biomaterials. In this fact, cells cultures in the presence of different types of biomaterials (pyrolytic carbon, cobalt-Chromium, titanium) has been performed. These cell cultures were subjected to biological characterization tests and mechanical characterization. The obtained results correlate with the in vivo observations (a promotion of the creation of a neocartilagical tissue in contact with the Pyrolytic Carbon implants).

1. Introduction

Degenerative shoulder pathologies associated with a functional rotator cuff are currently treated by anatomical total shoulder arthroplasty (TSA) or hemi-arthroplasty (HSA) [1].

However there is much concern of preservation of cartilage and bone stock and reducing occurrence of dislocation [2]. To face this issue, a new generation of spherical interposition implant has been developed. This implant is unique in the world in term of concept. Its design is based on the absence of skeletal embedding (contrarily to the TSA and HSA procedure), the implant mobility, the integrity of the tendons to stabilize the implant, the filling of the joint ensuring the effectiveness of the muscular lever arms, and the creation of joint surfaces with the two bone sections, humeral and glenoid.

Recently, the pyrolytic carbon (PyC) was selected as the implant material for this interpositional shoulder arthroplasty. This biomaterial has demonstrated an excellent tissue biocompatibility and good wear properties [3]. The proper functioning of this prosthesis is related to what is called "biological adaptability" especially at the PyC-humeral bone junction, which means creating of a painless biological tissue in contact with the implant [4]. The analysis of the explant, showed the creation of a biological “tissue” adherent to the bone. This tissue has the characteristics of cartilaginous tissue [5].

Thus, the behaviour of this implant against bone could regenerate cartilage and promote bone repair.

The creation of this “new cartilage” on the bone side, can be related to tribological effects (linked to the type of the biomaterial) or/and biological constraints (related to surgical bone preparation and the bone healing procedure).
The aim of this study is to identify the role of biological constraints (at the cellular level) surrounding tissues in the presence of different joint implant biomaterials.

2. Methods
Different primary cells cultures of murin chondrocytes and osteoblasts have been performed in the presence of different biomaterials (PyC, chromium cobalt CrCo, titanium Ti, and PolyEtherEtherKetone PEEK). These cells cultures were subjected to biological characterization tests (toxicity, viability, histology), surface topography analysis and mechanical characterization tests (adhesion force and rheology).

2.1. Biological characterization
In order to launch a cell culture, an implant sample disk-shaped (3 mm thick, 20 mm diameter) is placed in a cell plate with the corresponding cells. The cells are obtained after dissection on young mice (3-4 days-old) (chondrocytes source: femoral heads and knee cartilage; osteoblasts source: calvaria zone from skulls). For each type of cells minimum 3 cultures are scheduled in order to evaluate the repeatability of results.

After dissection, enzymatic digestion with Liberase is necessary to remove all soft and fibrous tissue [6]. During the culture of chondrocytes, two types of culture mediums were used: a complete medium composed by Eagle Dulbecco 4.5 g / L glucose + penicillin/streptomycin (1%) + L-Glutamine (20 mM) + foetal bovine serum (10%) + Hepes which helps the cells to adhere to the plates and a differentiation medium (50 µg/mL of ascorbic acid and 10 mM B-glycerophosphate) which stimulates the cells mineralization. On the 10th day of cells culture, viability and toxicity are analysed [7]. At the end of the cell culture the mineralization [8], the specific assay of calcium and phosphatase alkaline activity and the inflammatory reaction are analysed. Then the developed cells membranes are conserved for histological tests and mechanical characterization.

2.2. Surface topography and mechanical characterization
The surface topography of the biomaterial structure containing the cells membranes developed during the cell culture is analysed using scanning electron microscopy (SEM). The adhesion force of the cells is evaluated using an atomic force microscope (AFM) while their rheology is analysed using a bioreactor developed by LaMCoS research laboratory (pending patent). To do so, a dynamic compression test was applied to the cells membranes placed between a stainless steel tray and a plexiglas tray as schematized in Figure 1.

![Figure 1. Schema of LaMCoS bioreactor.](image)
3. Results and discussion
Until today, four independent chondrocyte culture tests have been done while osteoblasts culture tests are ongoing. The results have shown good cell viability for all types of studied biomaterials but a higher level of mineralization in the case of PyC. The assay of alkaline phosphatase activity has showed the same thing (Figures 2 and 3).

![Figure 2. Viability and mineralisation results.](image)

![Figure 3. AP Assay.](image)

The surface topography analysis by SEM (Figure 4) highlighted the presence of a cells membrane adhering to the biomaterial. Therefore, for the PyC substrate, the cells forming the membrane have a round shape and form more than one layer, compared to other substrates on which the cells are fewer in number, more flattened, hence more adhered to the substrate.

![Figure 4. SEM Visualisation. (a) Pyc sample and (b) PEEK sample](image)
Rheological analyzes are ongoing, the preliminary results have shown that the elasticity module of the cells membrane adhering to the PyC is higher than the one of cells membrane adhering to the CrCo.

\[ E(\text{PyC}) = 1.25 E(\text{CrCo}) \]

Histological analyzes are ongoing in order to further characterize these types of cells membranes obtained in vitro. Despite obtaining good results in terms of cell viability and non-toxicity of biomaterials, the biological experiments are not able to reproduce all the biological constraints in vivo. Concerning the mechanical constraints they may affect the cells mineralization, therefore the bioreactor tests are necessary to validate this hypothesis.

4. Conclusions
Preliminary results (creation of a cells membrane on the PyC surface) correlate well with the in vivo observations (creation of a neocartilagical tissue in contact with the PyC). In order to validate the role of this biomaterial in the creation of the new tissue, further investigations are required: Repeat the same tests with osteoblasts cells culture; Study the role of the mechanical and physicochemical stresses in cells behaviour in contact with the biomaterial: bioreactor tests (rheology and dynamic tests).

5. References
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