2012

P1

Activity 3.7 targeted the selection of cell culture system to distinguish and monitor chondrogenic diferentiation. To achieve these, P1 tested (i) different media inducing cell differentiation and (ii) different types of cell-culture system in two-dimensional (2D) and pellets.

The chondrogenic differentiation process was monitored for 28 days post induction in terms of: (i) the gene expression level by RealTime RT-PCR (Sox9, ColagenII, agrecan, CEP68 and CD151), (ii) the proteic expression level by Western Blot (Sox9, Collagen II and CEP68), (iii) the proteic expression level by immunofluorescence performed on sections of pellets at 14 and 28 days post induction was followed highlighting markers Sox9, collagen II, agrecan and CEP68.

Our conclusions are: (i) ADAS cells can differentiate into cells of "chondrocyte-like" type in standard culture conditions and under a strong inducer of chondrogenic environment. Differentiated cells resulted in the process are capable of producing cartilage specific extracellular matrix.

(ii) From the different media recipes for ADAS differentiation, we have chosen as the most efficient the MD2 composition.

(iii) The pellet cultivation system was designated as the most efficient.

(Iv) transcription factor Sox9 may be considered early chondrogenic marker, while collagen II, and CEP68 can be considered late markers.

P2

P3

In this phase **Partner P3 – University Polytehnica of Bucharest – Faculty of Applied Chemistry and Materials Science** has developed **polymeric scaffolds in the form of films and membranes, hydrogels or 3D porous networks** from various natural polymeric (collagen, sericin, hyaluronic acid, chondroitin sulfate) or mineral (nano-hydroxyapatite and active bioceramic provided by P7 partner) components. They were characterized by physical-chemical (FTIR, XPS), thermical (DSC and TGA/DTG), morphological (SEM, water up-take – kinetics and at equilibrum), mechanical analysis and also by biodegradability and by protein release. Among all the synthetised and characterized samples, P3 partner selected for biologial tests some types of polymeric scaffolds with appropriate properties in order to achieved the objectives proposed for year 2012.

Thus, in order to accomplish the **objective 1** (activities **1.1, 1.3, 1.5**) of the project, P3 has achieved several series of **scaffolds usable in bone tissue engineering**. After physical-chemical, thermal, morphological and mechanical analysis performed by P3 on polymeric scaffolds as films and membranes, hydrogels or 3D porous networks, 9 types of lyophilized superporous scaffolds were selected. These were based on **collagen** (1.2%), **collagen-sericin** (Coll: SS - 100:20 and 100:40) **collagen-sericin-hydroxyapatite** (Coll: SS: HA - 100:0:30, 100:

20:30 and 100:40:30) and **collagen-sericina-bioactive ceramic** (Coll: SS: HA - 100:0:30, 100:20:30 and 100:40:30) and were provided to the **P2 partner in order to evaluate the biological properties and their using for bone tissue engineering**.

Within **objective 2**, P3 has been involved in the activities **3.1, 3.2, 3.4 and 3.5** by the synthesis and characterization of some binary and / or ternary hydrogels which were provided to P1 in order to perform specific biological tests. Porous scaffolds in the form of hydrogels were based on four biopolymers: collagen, sericin, hyaluronic acid and chondroitin sulfate in various concentrations. Taking into account the results of analysis, 5 compositions of hydrogels were selected, as following: **collagen-sericin** (Coll: SS - 100:40), **collagen-sericin-hyaluronic acid** (Coll:SS:AH - 100:40:10 and 100:40 : 5) and **collagen- sericina-chondroitin sulfate** (Coll:SS:CHS - 100:40:10 and 100:40:5). The 3D hydrogels were sent to P1 partner to assess biocompatibility properties and their using for cartilage tissue engineering.

Some of the results were disseminated by **2 published ISI** articles (impact factor 3,975), a **book chapter** sent to be published, the participation with **3 papers at international conferences / congresses** and **2 papers at the PCCE workshop**.

The activities according to the implementation plan have been totally fulfilled.

P5

1.5.*d.* Establishment by electrochemical methods of the long term behaviour of the films obtained on supports of Ti and TiAlV

Cyclic voltammetry curves prove the improvement of the in time biomaterial behaviour, namely the HA formed layer becomes more compact and thickens in time, thus is more bioactive.

Nyquist spectra show a capacitive behaviour, a very resistant protective layer. The impedance increases with the immersion time, so, the HA film thickness also increases.

1.5.*g.* Long term morphological characterisation of the activated surfaces on supports of Ti and TiAlV by SEM

SEM study revealed the thickening of the HA coating on the Ti and Ti-6Al-4V alloy surface after immersion for 3000 hours in physiological solutions. The coatings compacted and had an homogeneous aspect, indicating a thick bi-dimensional layer with round particles and pores of about 100-250 nm diameters.

1.5.*j.* Medium term behaviour by electrochemical methods of the obtained films on support of TiNbZrTa alloy

For the covered alloy, all electrochemical parameters became more favourable in time as result of the deposition of new protective HA layers. EIS spectra confirm the ability to stimulate the formation of HA, so high bioactivity, osseoinduction and osseoconduction of the coating.

1.5.*l.* Medium term morphological characterisation of the surfaces activated on support of TiNbZrTa by SEM

Important changes of the coating morphology resulted after 2000 h; HA nanocrystales became spherically with a spongy morphology with diameters ≈ 60 nm, indicating a process of gradual growth of HA.

P7

The aim of the studies was to design and to get new pH and thermo –sensitive hydrogels for cartilaginous tissue engineering based on a natural bio-poly-amino-saccharide, chitosan. In this connection, the possibility to alter the chitosan solution hydrophilic - hydrophobic balance, in order to generate a sol-gel transition at physiological temperature of 37 °C and pH ranged between 6.9 and 7.4 in aprox.3 hours were studied and indentified. The new hydrogels had to fulfill the following conditions: to have a variable consistency, from those to be not miscible with the culture medium to those to be handled, to be not cytotoxic, to have a homogeneous porosity and mechanical properties allowing their use as 3-D scaffold in cartilage tissue engineering. The chitosan protonation and neutralization possibilities in order to generate, in solution, the sol – gel transition at 37°C and pH = 6.9 - 7.4, in the performed studies were identified.

The selection of new hydrogels in several steps including testing the hydrogels behavior as 3 – d scaffolds was considered. In the first selection stage, all the hydrogel obtaining variants at which the sol - gel transition does not occur at 37 °C and a pH between 6.9 and 7.4 and the hydrogels type of which consistency was outside of the interest limits were removed. The obtained results at biological tests have shown that the efficiency as 3 –D scaffolds is conditioned by the following main parameters: acid type with which the protonation of chitosan solution was made, the acid concentration, the removal degree of small molecular components from the polymeric solution, the type and concentration of neutralization salts, the morphology of the obtained hydrogels, the method to achieve the desired level of mechanical properties.

Other important selection criteria were: the homogeneity of the pore micro architecture and the hydrogels elastic properties. Based on chosen procedures for desired morphology realizing, hydrogels with spherical, interconnected pores and with size below 100 µm were realized. Thus obtained hydrogels have had 38 – 40 % swelling ration, 83 – 85 % hydration degree and have provided good properties as 3 –D scaffolds for cell growth and proliferation.

Studies in order to contribute to the reaction mechanism elucidating and to control the morphology and elastic properties for effectively appropriate hydrogels properties to cartilage tissue requirements there are in progress. The biological tests were realized by the project coordinator with whom P7 have cooperated very well for the entire studies period.