Poster

CALCIUM UPTAKE BY NANOHYDROXYAPATITE CRYSTALS, IN COMPOSITE MATERIALS WILL CHANGE CELL CULTURE MEDIUM PROPERTIES.

Jose Iñaki Alava, Beatriz Olalde, Maria Jesus Jurado, Nerea Garagorri.
Health Unit, INASMET-Tecnalia, Paseo de Mikeletegi nº2, San Sebastian, 20009, Spain
ialava@inasmet.es

Introduction.

During the development of composite materials for cell proliferation and grow, selection of nano or micro fillers is a key question. Many authors have reported anomalous response of cells in culture when used nanohydroxyapatite (1,2,3). This work shows the effect of hydroxyapatite crystal size on cell culture medium properties when the composite scaffold is exposed.

Material and Methods.

Scaffold Production. In this process, the nanoparticles: single walled carbon nanotube (SWNT) and nanohydroxyapatite (nHAp) or microhydroxyapatite (mHAp) are dispersed through out the solvent, made of Dioxane (Dx) and water (87/13), in an ultrasound bath and over 30 minutes. Then, the poly(L-lactic acid) (PLLA) is added and the suspension is sonicated at 65ºC until the polymer is completely dissolved (3 hours). Finally, the temperature of the solution is quickly decreased to induce phase separation, that is, to form 2 separated phases: One having a higher polymer concentration (polymer-rich phase) and a second phase that is lower in polymer concentration (polymer-lean phase). After the solvent has been removed by sublimation, the polymer rich phase solidifies into the skeleton of the scaffold, and the spaces occupied by the solvent in the polymer-lean phase become the pores of the polymer foam. Scaffolds with these fixed characteristic were manufactured: Quenching temperature (Tq: -16ºC); Solvent ratio (Dx/H2O: 87/13); And the process variables were: the percentage of HAp and the grain size of HAp particles .Cells and culture medium. Osteoblast (ATCC, CRL-11372) cells have been used. A unique culture medium was used for the osteoblast (ATCC, CRL-11372). Each 200 ml of culture medium contained: 20 ml foetal bovine serum (FBS), 2ml Penicillin (1% h/v), 1080 µl Gentamicine (0.5% h/v), 2ml Glutamine (1% h/v), 174.92 ml MEM-F12 de ATCC. All the scaffolds were sterilized by Ethylene Oxide. Assay methodology After the sterilization process, all scaffolds were immersed in the culture medium at 37ºC for 1 week. The material-culture medium ratio was, approx., 20mg of scaffold per 1ml of medium. The same culture medium was used through out the week (it wan not replaced) in order to maximize the reactivity. Finally, after periods of 24h, 48h, and 6 days, the samples were removed from the culture medium and both the scaffold and the medium were characterized. Culture medium analysis. Determination of pH changes, samples were kept in culture medium at 37ºC. After 24, 48 hours and 6 days, the scaffolds were removed from the culture medium and the pH of the medium was tested electrochemically at 37ºC with partial pressure of CO2 compensation. (4). Evaluation of ions content was done, in the culture medium (calcium, phosphate, phosphorus, magnesium) by Inductively coupled plasma-atomic emission spectrometry (ICP-AES) (4). Cytotoxcity after a cell-medium contact of 24h, cells were collected to put in contact with the extract of the sample during several hours. After that, the quantitative evaluation (staining collected cells with 7-Amino-Actinomycin D and quantifying the number of cells by a flow cytometry method (TruCOUNT Tubes, Beckton & Dickinson) of cell cultures were made.

Results

Fig (1). Firstly, when the percentage of hydroxyapatite increases from 1% to 10%, the effect on the pH is bigger, it decreased more. For example, the scaffold behaviour made of 10% of nanoHAp when kept in contact with the culture medium, induced a pH decrease of 7.40, but when 1% of the same CaP was used, this value was 7.43.And also, the effect of micro size HAp (mHAp) on the pH values is lower than the effect of the nano sized one (nHAp), due to the bigger grain size. For example, over 6 days, the pH of ref.10% nHAp was 7.40, while the

pH of the ref.10%mHAp was 7.42. Secondly, the nanoparticles dispersion into the foam could affect to the pH of the culture medium. Focusing on the samples 10% nHAp, where the scaffold composition was the same and the principal difference was the nanoparticles mixing method, the samples 10% nHAp shown a bigger decrease from the first stages. And all of this happens despite the fact that the change in the pH over the 6 day-period was similar. This behavior should be due to the poor dispersion of the nHAp into the foam. In general, Ca and P concentrations decreased in all culture medium that have been in contact with the scaffold. The scaffolds made of low percentage of nano and microHAp, 1%, show a slight decreased of these ions contents in the culture medium. However, the sample with 10% of nHAp or mHAp present a higher decreased. The culture medium that has been in contact with the scaffold made of 10% of nHAp the Ca and P concentration decreased down to 44 and 26 mg/l, and when the scaffold has 10% of mHAp, these values are 58 and 31 mg/l, respectively Fig. (2). On the other hand, the lowest obtained values for the percentage of the growth of cells are 70% (in the case of the 1% mHAp sample after 24 hours of lixiviation) and 77% (in the case of the 10% nHAp sample after 24 hours of lixiviation). In anyway, these percentages are not low enough to consider cytotoxic extracts for the bone cells, but one small inhibition of cell grow will be observed.

Conclusion.

This confirms that the presence of high nanocrystalline HA percentage, depletes the calcium and phosphorous and the effect of micro size HAp is lower due to the bigger grain size. This effect is attenuated when the percentage of HAp is lower. It can be concluded that the tested extracts of the samples are non-cytotoxic and there is no enough difference between the cytotoxicity of them. This work has been supported by EC inside Nanobiocom Project –NMP3-CT-2005-516943.

References:

(1) Xiaoming Li, Qingling Feng, Fuzhai Cui. Materials Science and Engineering **C 26** (2006) 716 – 720.

(2) Sang-Soo Kim, Min Sun Park, So-Jung Gwak, Cha Yong Choi, and Byung-Soo Kim. Tissue Engineering, **12**, Number 10, 2006.

(3) Sánchez-Salcedo S, Izquierdo-Barba I, Arcosand D, Vallet-Regí. M. Tissue Engineering **12**, 279-290 (2006)

(4) Standard Methods for the Examination of Water and Wastewater 20th edition

Figures

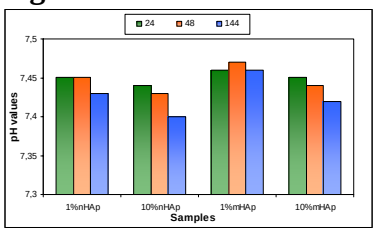

Fig.1: Evolution of pH of culture medium as a function of time in contact with different scaffolds

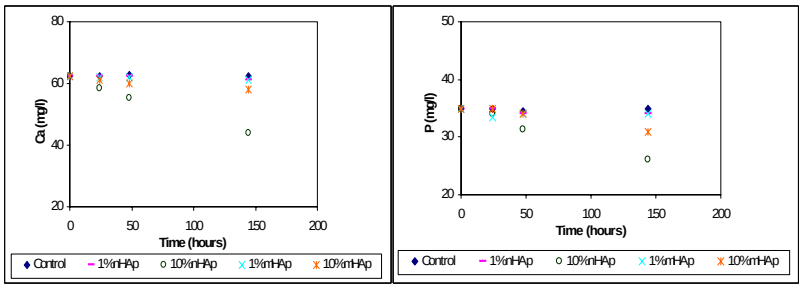

Fig.2: Evolution of Ca and P content of Medium in depended of the scaffold kind

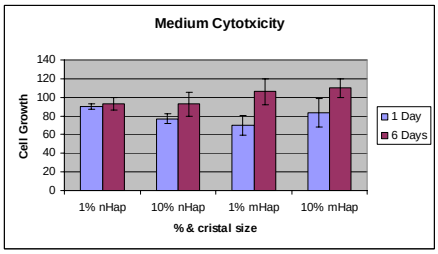

Fig 3: Induced cytotoxicity in the medium