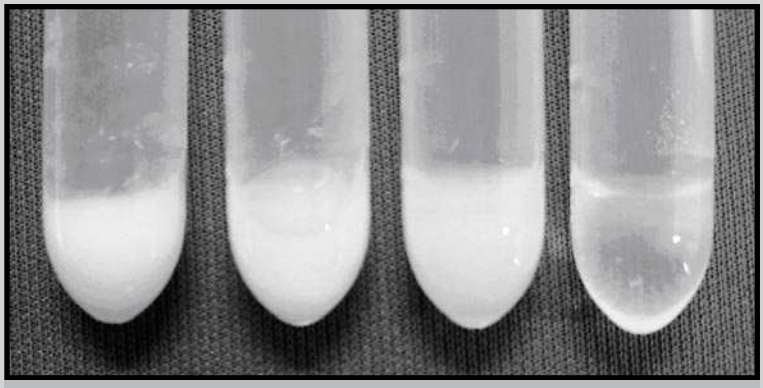

FIGURE 1. Collagen hydrogels containing calcium phosphate (CaP) nanoparticles. CaP-collagen mass ratios from left to right: 4:1, 2:1, 1:, control without CaP).

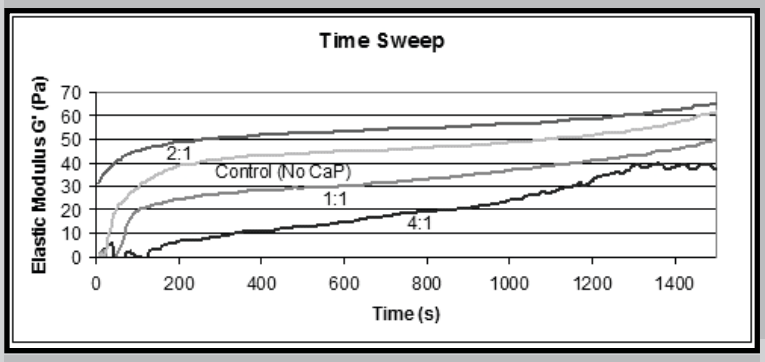

FIGURE 2. Time sweeps showing speed of collagen hydrogel formation at different CaP-collagen mass ratios.

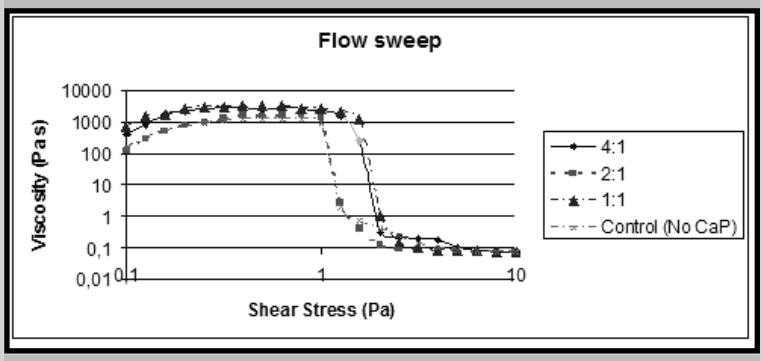

FIGURE 3. Flow sweeps showing viscosity of collagen hydrogels at different CaP-collagen mass ratios as a function of shear stress.

CaP suspensions were added during gel formation instead of ddH_2O. Rheological characterisation of the speed of gel formation (time sweep) and the mechanical properties of the gel after formation (flow sweep) were carried out using a AR2000ex Rheometer (TA Instruments) at 37°C.

Results and discussion

It was possible to distribute CaP-particles at CaP:collagen mass ratios of 4:1, 2:1 and 1:1 (FIGURE 1) Time sweeps (FIGURE 2) showed no remarkable differences in the kinetics of gel formation between controls without CaP and CaP-collagen mass ratios of 2:1 and 1:1, however gel formation was slower at 4:1. Flow sweeps (FIGURE 3) showed similar patterns for controls and all CaP-collagen mass ratios. These results show that incorporation of CaP particles does not have a negative effect on gel mechanical properties.

Summary and future work

This work showed the feasibility of incorporating CaP particles into collagen hydrogels during gelation without adversely affecting the mechanical properties of the gel. Future work will concentrate on the effect of CaP incorporation on the ability of collagen gels to be mineralised and biocompatibility studies.

Acknowledgements

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BLOOD PLATELETS APOPTOSIS IN HEMODIALYZED PATIENTS

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Abstract

Blood platelet proteome of hemodialyzed uremic patients exhibits significant difference in comparison to the blood platelet proteome of healthy subjects. This alteration is manifested by the presence of high concentrations of low molecular peptides within the whole range of pI. Increased platelet apoptosis has been put forward as a possible cause of this phenomenon (1). The aim of the present research was to assess whether blood platelet populations from hemodialyzed uremic patients exhibit more binding sites for Annexin V (a marker of apoptosis) than control samples from healthy donors. Blood was obtained from uremic patients immediately before and after hemodialysis. At the same time samples from control healthy donors were also collected. Blood was anticoagulated with sodium citrate and was immediately exposed to propidium iodide,

fluorescent labeled Annexin V and CD61 antibodies. The samples were incubated for 10 minutes in the dark and next the labeled samples were processed in a BectonDickinson FACScan flow cytofluorymeter. Our preliminary study was performed for 12 hemodialyzed patients, 13 nondialyzed uremic patients and 12 controls. It was found that the blood platelet population of hemodialyzed patients exhibited significantly higher level of fluorescence intensity attributed to Annexin V. Furthermore, this intensity was comparable before and after hemodialysis and was independent on patient age. The results support the hypothesis that blood platelet contact with artificial surfaces during the process of hemodialysys may be partially responsible for triggering blood platelet apoptosis.

[Engineering of Biomaterials, 89-91, (2009), 29-30]

Acknowledgement

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ENDOTHELIAL CELL PROTEOME CHANGED BY CONTACT WITH SURFACES OF BIOMATERIALS

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Abstract

Biomaterials used for medical implants or instruments production can cause numerous undesirable effects in human organism. They may affect cells being in a direct contact with them and can cause changes in genes expression, and as a consequence, also in protein profile of these cells. The aim of the present work was to examine an effect of medical steel 316L, poly-para-xylylene (Parylene) and nanocrystalline diamond (NCD) surfaces on protein expression in human endothelial cell line EA.hy 926. Cells were grown in Dulbecco's MEM (DMEM) supplemented with antibiotics (penicillin and streptomycin), glucose, 10% heat inactivated fetal bovine serum and HAT-supplement. After 48h of incubation cells were washed with PBS and treated with lysis buffer (7M urea, 2M thiourea, 4% CHAPS, 2 % IPG buffer pH 4-7, 1% DTT). Proteins were purified from cell lysates with 2-D CleanUp Kit, and concentration was assessed with 2D Quant Kit. After overnight rehydration of IEF strips (pH 4-7, 11cm), in the presence of purified proteins, isoelectric focusing procedure was performed until 40kVh. Then, stripes were equilibrated, and focused proteins were separated in 12,5% polyacrylamide gels (SDS PAGE). Silver stained gels were recorded with ImageScanner and analyzed with ImageMaster 2D Platinium 6.0 (GE Healthcare) software. Numerous changes in protein expression were detected in endothelial cells exposed to artificial surfaces of tested materials (see TABLE I).

[Engineering of Biomaterials, 89-92, (2009), 30]

Biomaterial	Total number of detected spots	Number of matched spots	Number of over-expressed spots	Number of suppressed spots
Medical steel 316	301	187	45	66
Parylene	283	164	59	54
NCD	423	224	38	75
None (control)	339	339	-	-

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NANOSTRUCTURE OF BOVINE PERICARDIUM TREATED WITH TRYPSIN

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Abstract

Various methods of xenogeneic tissues stabilization have been proposed for the purpose of preparing many tissue-derived biomaterials. One of the most important treatments that may lead to obtaining the good-quality tissue biomaterials seems to be decellularization of such tissues. This process may contribute to the reduction of the most frequent failures resulting from the tissues stabilization. The aim of this work was to determine nanostructure of trypsin-treated bovine pericardium, using atomic force microscopy (AFM). The treatment of bovine pericardium with trypsin in EDTA solution resulted in non significant changes in tissue's morphology. Demonstrated AFM studies of these tissues revealed no failures on the fibers' surface in the nanoscale. Thus, our results confirm the expectation that decellularization may be considered as one of the most promising methods of the allogeneic and xenogeneic tissues stabilization.

Keywords: *nanostructure, bovine pericardium, collagen fibers, trypsin*

[Engineering of Biomaterials, 89-91, (2009), 30-32]