BIOCOMPATIBILITY OF C-C COMPOSITES COVERED WITH PyC AND pHEMA

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Introduction

The application of carbon-carbon composite materials as a biomaterial is mainly limited by its cost and brittleness of the matrix. The brittleness leads very often to the formation of microparticles in the tissue, which may cause inflammations around implants. [1,2] To prevent the releasing of carbon particles, C-C composites have been covered by different layers. In our work we have studied the biocompatibility of C-C composite surface covered with pyrolytic carbon and pHEMA - poly(2-hydroxyethyl methacrylate) with biopolymer collagen, synthetic polymeric hydrogel utilized for biomedical applications. [3]

Samples preparation

The specimens were reinforced with the carbon plain wave fabric from Torayca T800H fibres with the phenolic resin Umaform LE as a matrix precursor. The specimens were three times impregnated and recarbonised in the N_2 atmosphere at 1000°C, and infiltrated and covered with pyrolitic carbon. Final carbon-carbon samples were impregnated and covered with pHEMA solution in the autoclave by changing of vacuum and pressure of 0,4 MPa at 40°C, and then dried in vacuum at 50°C for 24 hours.

Measurement methods

1. Microscopy and image analysis
2. Infrared microspectroscopy
3. Biocompatibility in vitro tests

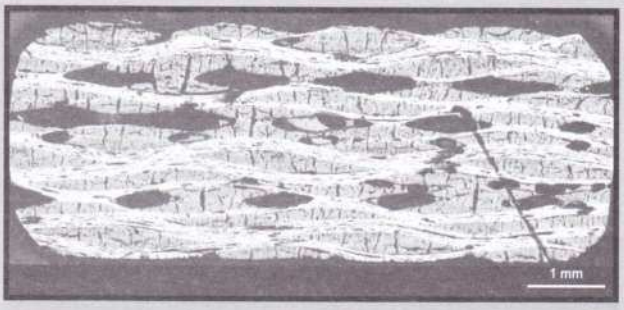

FIG. 1. Micrograph superposed from 20 image fields.

Microscopy and image analysis

For the measurement, polished sections were prepared. Sample was embedded in epoxy resin and the exposed surface first smoothed, and then polished to a scratch- and relief-free finish.

Rate of pores, open pores and pores infiltrated with pHMA were determined by the image analysis method in the system LUCIA G version 4.60, Laboratory Imaging Ltd, Prague, Czech Republic, using the metallurgical microscope Nikon Optiphot 100S, and the colour CCD camera Hitachi HV-C20 connected with the frame grabber FlashPoint Intigue Pro.

FIG. 2. Image analysis equipment.

For the maximal differentiation of composite mass and pHEMA the initiation macroprogram was prepared that adjusted the intensity of illumination by gain setting and white balance on camera and the aperture stop of the microscope. For grabbing itself the shading correction was used. The quality of micrographs significantly affects further measurement. Micrographs of the sample surface and for measurement are shown at FIGS. 3,4.

FIG. 3. Micrograph of the sample surface (magnification, 50x).

The colour images were converted into the grey scale with the further contrast enhancement and into the segmented binary image suitable for the measurement than. All pores in composite materials were measured as a first step. The field measurement was used to measure area fraction of all voids in the composite mass. Area fraction value is defined as the ratio of the segmented image area and the measured area, it has a strong stereological interpretation: in the case of isotropic uniform random sections it is equal to the volume fraction.

Pores uninfiltrated with PyC were erased than and area fraction of open voids (pores and cracks) was measured. The same step was used to erase the pores not penetrated with pHEMA; the third area fraction measurement was the last step of image analysis.

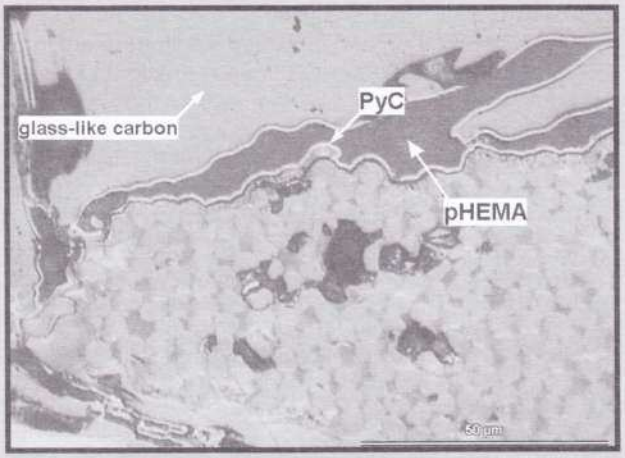

FIG. 4. Micrograph for measurement (magnification 500x).

Infrared microspectroscopy

The infrared microspectra were measured using Magna 750 spectrometer (Nicolet Instruments Co., USA) coupled with IR Plan microscope (Spectra Tech) with an MCT detectore. 1024 scans at resolution 2 cm^{-1} were used. The spectra were scanned in the reflection mode from the polished sections with measuring area of 50 x 50 μm. The resulting reflectance spectrum was expressed in Kubelka-Munk units using Omnic Nicolet software. Microspectrum of the pure pHEMA polymer was measured as a film on a metal plate. The infrared spectrum of the pHEMA polymer used is shown in FIG. 5. Collagen (0.5 %) present in the sample is under the detection limit of the technique used.

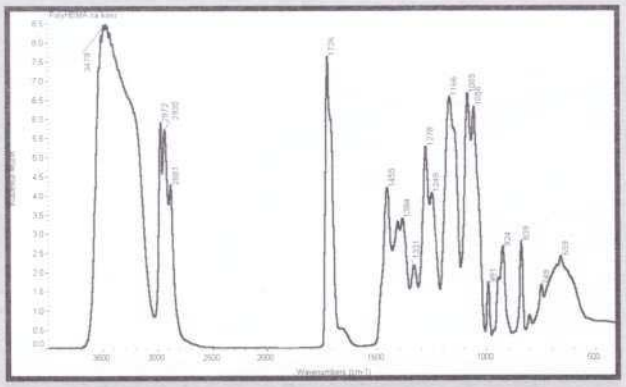

FIG. 5. The pHEMA infrared microspectrum.

In vitro test

Embryonal human lung fibroblasts were cultured on composites for three days at 37°C in 5% CO_2 atmosphere. Plastic Petri dishes for tissue culture were taken as a control surface. The metabolic activity of cultured cells, and the level of some cytokines were determined.

The effect of biomaterials followed on the production of cytokines in the culture of embryonal human lung fibroblasts was evaluated. Cytokines are low-molecular-weight peptides used by immune and inflammatory cells to communicate with each other. In this study we evaluated the expression of proinflammatory cytokines tumour necrosis factor - α (TNF-α) and interleukin - 8 (IL-8). TNF-α is a potent paracrine and endocrine mediator of inflammation. IL-8 is a cytokine, which mainly functions as a neutrophil chemoattractant and activating factor. Its expression is stimulated by TNF-α. Both cytokines are secreted by activated monocytes, macrophages and many other cells including fibroblasts.

	Volume fraction in all composite mass	Respective volume fraction between each measurement step
Voids (pores and cracks)	27,82%	27,82%
Voids infiltrated with PyC	16,28%	58,5%
Voids penetrated with pHEMA	9,28%	57%

TABLE 1. Image analysis results.

Results and discussion

Microscopy and image analysis

The volume fraction of pHEMA in inner pores of composite was detected from polished cross-sections by image analysis method. 27,8 % was volume fraction of coarse pores in sample, 58,5 % of all pores stayed open and were infiltrated with PyC, and 57% of those pores were penetrated with pHEMA.

Infrared microspectroscopy

Analysed areas of the sample are marked on micrograph in FIG.6 and their spectra are depicted in FIG.7. Following analytical bands have been selected from the spectrum:

~3400 cm^{-1}	OH groups
2930, 2860 cm^{-1}	aliphatic C-H bonds
1730 cm^{-1}	C=O
1459 cm^{-1}	aliphatic C-H
1166, 1085, 1057 cm^{-1}	C-O
841, 752 cm^{-1}	aromatic C-H,

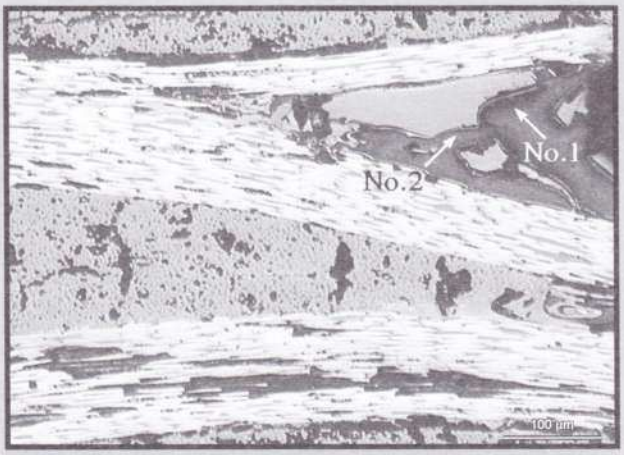

FIG. 6. Micrograph of the sample with the analysed areas marked.

which do not interfere with the absorption bands of the surrounding matrix and thus are suitable for determination in the matrix of the composite. Then, these absorption bands have been found in the spectra of the given analysed areas. Their existence confirms with a great probability of presence of the pHEMA in the polished sections of the analysed samples.

In vitro test

The cells cultured on C-C composites coated with pHEMA exhibited three times higher metabolic activity in comparison with the uncoated C-C composite.

The levels of both inflammatory cytokines on C-C com-

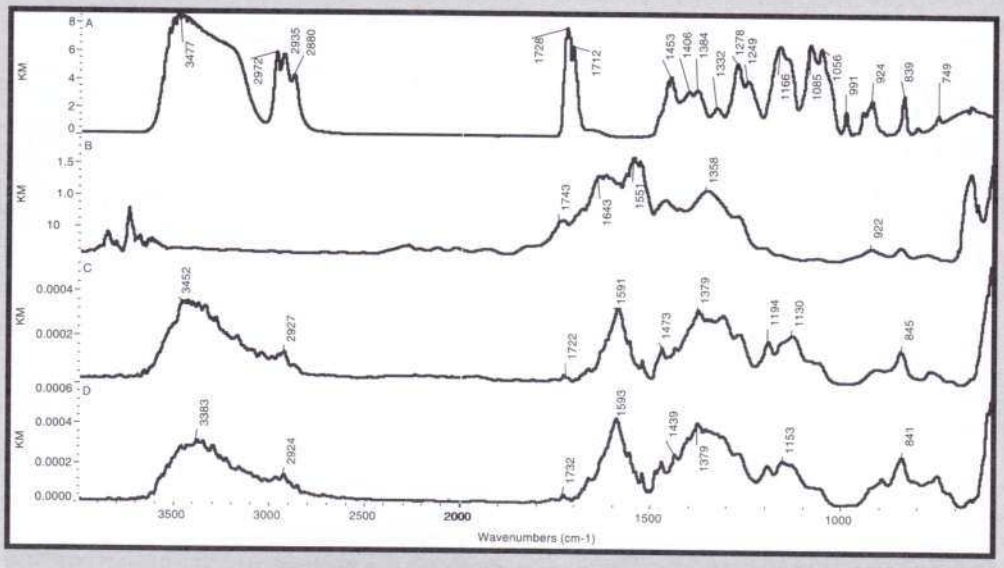

FIG. 7. Infrared microspectra of the pHEMA polymer, glass-like carbon, and analysed areas marked in FIG. 6.
A - pHEMA, B - glass-like carbon, C - analysed area No. 1 , D - analysed area No. 2

posites were higher in comparison with the control surface. There was not significant difference in both inflammatory cytokine levels between the samples of C-C composite and C-C composite with pHEMA.

Sample \ Cytokines	IL – 8 ± SD (pg/ml/1 cm^2)	TNF – α ± SD (pg/ml/1 cm^2)
C-C composite	594,3	0,914
C-C composite + pHEMA	587,9	1,017
Control	206,1	0,303

TABLE 2. Cytokines concentration on 1 cm^2 of cultivation medium.

Conclusion

The presence of pHEMA on a surface and inside the pores of studied C-C composite was confirmed with the used methods.

In vitro tests proved three times higher metabolic activity of cells cultured on C-C composite with pHEMA in comparison with the control surface. There was not significant difference in inflammatory cytokine levels between the samples of C-C composite and C-C composite with pHEMA.

Acknowledgements

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[2] Burešová, M., Balík, K.: Study of the surface properties of C-C composites as biomaterials, Engineering of Biomaterials, 12, (2000), 28-30
[3] Tan, J. M.: An overview of pHEMA Properties, Applications, and Future progress, Biomaterials 4/24/2000