# SOME IN-VITRO BIOCOMPATIBILITY TESTS OF CARBON – CARBON COMPOSITES AND PREPARATION OF BONE PLATES

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## Introduction

Carbon is known to have the best biocompatibility of all materials[1]. This material is compatible with blood, bones and tissue. Carbon – carbon composites (CFRC) can be manufactured in such a way as to posses mechanical properties identical to properties of bone, thus eliminating the need for the implant removal once the healing is completed.

The use of this material is limited by difficulties of effective stress analysis, its cost and its brittleness. The brittleness leads very often to the formation of microparticles in the tissue, which may then cause inflammation around the implants [2, 3, 4, 5].

To avoid this problem, we tested "in-vitro" various samples of 2-D composites. The further aim of this study was to suggest the design of bone plates and to compare the different reinforcement of this material.

## Materials and test methods

Three types (A, B, and C) of composites reinforced by plain-weave carbon fabric (made of Torayca T800 carbon fibre) and with a matrix derived from phenolic resin were manufactured using the prepreg technique.

The cured samples were carbonised at the heating rate of 50°C/hr up to 1000°C in the nitrogen atmosphere. One-step impregnation with pure phenolic resin and re-carbonisation yielded the material A. The material B was obtained by graphitisation at 2200°C in argon. Some graphitised samples were further coated with pyrolytic carbon at 1400°C, which resulted in the material C.

Two cytotoxicity tests of materials A, B, and C were performed using mammalian cells:

1) Four repeated extracting procedures (deionized water, 121°C, 130 kPa, 1 hr) which simulated conditions more severe than those in living organisms, in order to determine the toxicological hazard. These extracts were added to the culture medium with fibroblasts.

2) Indirect contact test, which studied the influence of released microparticles from the tested materials on the morphology of macrophages.

For the preparation of bone plates we have used the same fabric and resin as above. The reinforcement of composites was built by stacking, coiling, or by a combination of both. The curing was carried up at 0.6 MPa and 130°C in autoclave in a silicon rubber mould. All cured plates were carbonised at 1000°C in nitrogen. The open porosity and apparent density were measured by water penetration method according to the ASTM-C 20 standard. The structure of reinforcement was studied by an optical microscope.

| Samples | $V_f$ [%] | Density (g/cm$^3$) | Open porosity (%) | Flexural strength (MPa) | Flexural modulus (GPa) |
|---|---|---|---|---|---|
| A Carbonized | 47 | 1,4 | 22 | 97±12 | 26±3 |
| B Graphitized | 48 | 1,5 | 12 | 229±17 | 55±5 |
| C Graphitized, covered by pyrolytic carbon | 48 | 1,54 | 9 | 231±23 | 52±5 |

**TAB. 1. Properties of the investigated composites**

## Results and discussion

The properties of the tested materials are summarised in the Table 1.

### Extraction test

Although sterilisation conditions which are more gentle with regard to the investigated material are known (sterilisation by gases, sterilisation by ionising radiation), the test in question was chosen in order to reveal – under conditions which are several times more drastic than those existing in a living organism – the toxicological hazard caused by the investigated materials as well as potential changes in the mechanical properties of the materials caused by the conditions of

| Extraction (absorption 492-580 nm) | | | | |
|---|---|---|---|---|
| Samples | 1st extraction | 2nd extraction | 3rd extraction | 4th extraction |
| A Carbonized | 379 | 305 | 306 | 309 |
| B Graphitized | 360 | 313 | 322 | 321 |
| C Graphitized, covered by pyrolytic carbon | 380 | 311 | 308 | 311 |
| Standard ( pure plastic plate) | 357 | 325 | 326 | 324 |

**TAB. 2. Proliferation of the cells in the extracts from the tested materials**

the test. The mechanical properties given in Table 1 have not changed after the extraction test.

Table 2 shows the proliferation of the cells in the extracts. The higher is the value of absorbance (see Table 2) the higher is also the stimulating effect of the extract on the cell proliferation. The first extract is – from the point of view of application of the material in the living organism – the most important one. From Table 2 it is evident that the extracts from material A (carbonised) and from the material C (graphitised and coated with pyrolytic carbon) exhibit in comparison with the standard (pure plastic plate) a slightly stimulating effect on the proliferation of the cells. The effect of the subsequent extracts practically does not change and in comparison with the standard those extracts exhibit a slightly inhibiting effect.

The groups of cells from all extracts are metabolically active, which means that the materials A, B, D are not toxic to the proliferation of human cells.

### Indirect contact test

We utilised the ability of the macrophages to absorb the released particles of the materials and we evaluated the three materials in question according to the quantity of the absorbed particles.

From Table 3 it follows that the smallest amount of absorbing cells in their typical form and thus the lowest quantity of released particles was observed with the material C. This is proved also by the increased number of macrophages in the fibroblast-form that appears always when the number of particles is low.

The materials in question exhibit an amount of the last, blastic forms that is lower than that found with the standard; this proves the biocompatibility of all materials.

### Design of the bone plates

The design of the fixation plates is shown in Fig. 1. The shape was suggested to match the shape of the pig femur. The plates are 65 mm long, 15 mm wide, and 4.5 mm thick. They contain six holes with a diameter of 3.7 mm.

### Comparison of plates with various reinforcement types

The properties of plates prepared with various reinforcements are given in Table 4.

It follows from Table 4 that the bone plates manufactured by a combined stacking – coiling method reveal the minimal open porosity. Simultaneously they possess the highest density. According to Fig. 2 the plate manufactured by stacking contains matrix-rich regions (without fibres) in locations next to the bone. After fixation these regions are supremely mechanically loaded. On the other hand, the plate manufactured by coiling contains near its centre a large amount of voids and pores.

However, the bone plate made by a combined method (with a stacked core and coiled sheath) does not contain voids inside and the carbon fibres are homogeneously distributed within the cross-section. The coiled structure moreover suppresses the tissue irritation by free ends of the fibres, which is another advantage of the latter design. Eventually, the manufacture of the combined plate is at hand lay-up easier than the stacking method.

## Conclusion

Graphitised and coated by pyrolytic carbon C-C composites reveal after the autoclave treatment a good stability of mechanical properties and biocompatibility of its extracts. This material releases also a minimum amount of carbon particles.

The bone plates manufactured by the stacking/coiling method possess a homogenous reinforcement in the whole cross-section and contain negligible amount of voids and pores.

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| Macrophages (%) | | | |
|---|---|---|---|
| | Forms | | |
| | Typical | Fibroblast-like | Blastic |
| A Carbonized | 70 | 29,5 | 0,5 |
| B Graphitized | 71 | 28 | 1 |
| C Graphitized covered by pyrolytic carbon | 67 | 32,5 | 0,5 |
| Standard (pure plastic plate) | 66 | 32 | 2 |

**TAB. 3. Morphological evaluation of cells cultured in the presence of tested materials**

| | Stacking | Coiling | Stacking/coiling |
|---|---|---|---|
| Open porosity (%) | 33 | 36 | 29 |
| Apparent density (g/cm³) | 1,21 | 1,17 | 1,27 |
| Linear shrinkage (%) | 11,7 | 9,6 | 15,6 |

**TAB. 4. Properties of the plates after carbonization**

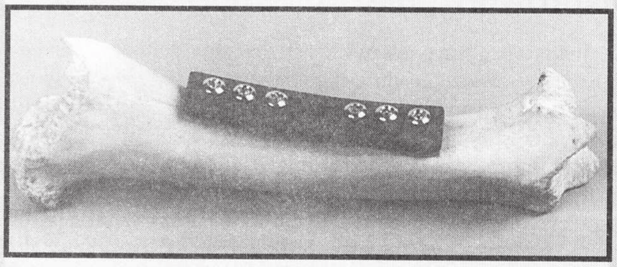

**FIG. 1. The bone plate fixed on a pig femur**

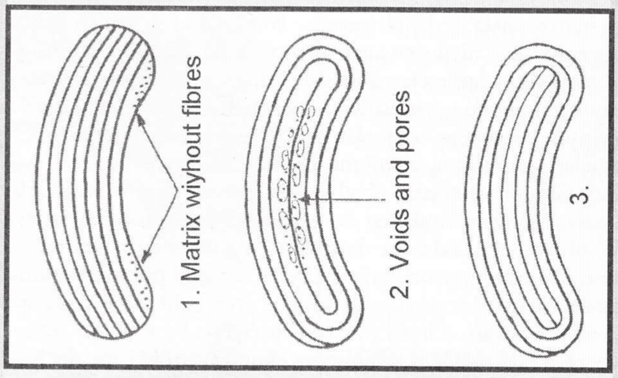


**FIG. 2. Schematic drawing of cross-sections of the plates prepared by: 1-stacking; 2-coiling; 3-stacking/coiling**

## References

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