An *in Vitro* Study of Polymer-Based Composites

Bożena Konieczna*, Marta Błażewicz*, Barbara Czajkowska**

ANNALS OF TRANSPLANTATION, Vol. 9, No. 1A (Suppl.), 2004, pp. 68–71

Abstract:
Structures of most tissues in the human body can be simulated with fibrous composite materials. A major problem associated with designing biocompatible composites for reconstruction of damaged or missing tissues is the ability to mimic such structures. The physical, chemical and mechanical properties of composite materials should be similar to those of the native tissue. Another very important factor of polymer-based fibrous composite materials, which can relatively easily be modified, is their surface microstructure. This surface microstructure depends on the way of preparation, type of polymer matrix and kind of reinforcement.
This work was aimed to determine the biological properties of composites obtained from carbon fibres and a polymer matrix, which can be used as biomaterial in the reconstruction of cartilage tissue. Two types of samples made from short carbon fibres and two kinds of polymers were tested. The samples were prepared by casting technique. MTT tests were carried out in the presence of hFOB-1.19-line human osteoblasts and HS-5-line human fibroblasts. The results show differences in viability of living cells.
Results of the work show significant differences in biocompatibility of pure polymers and composites with short carbon fibres.

Acknowledgements
The work was supported by grant No PBZ/KBN/082/T08/2002.

Key words: Polymers; Carbon Composites; Cell Response

Introduction

The medical use of synthetic polymers has a long history. On the basis of many laboratory and clinical investigations, there are polymers, which are considered as biocompatible [1], for example: polyethylene (PET), polypropylene (PP), polytetrafluoroethylene (PTFE) and polysulfone (PSU). These polymers are also biostable in the human body and have found wide applications in the medical field.

One of the polymers mentioned above, polysulfone, possesses many favourable properties, like resistance to oxidation and hydrolysis as well as excellent stability in aqueous inorganic acids, alkalies and saline solutions [2]. Another outstanding property is its inertness in the living body, enabling it to be used in practical devices such as medical bottles, respiratory sets and dialysis membranes [3-5].

Several other polymers have also been investigated, i.e. polyvinylidene fluoride (PVDF) with improved textile and biological parameters [6]. This polymer is more resistant to hydrolysis and degradation in comparison with PET. It has also been used in vascular surgery for many years due to its improved biostability and minimum tissue response [7]. PVDF is used in soft tissue applications and as a suture material [8, 9].

The confirmed biocompatibility of such polymers is a feature which allows them to be combined with carbon fibres to obtain a biocomposite. Such a composite promotes living tissue interaction due to advantageous influence of carbon fibres on the living body, previously presented in literature [10, 11]. Carbon fibres have been applied in various fields of medicine. Carbon strands and braids are used to reconstruct ligaments and tendons, whereas carbon fabrics are used in the treatment of tissue defects or as compounds in tissue engineering. Carbon fibres have proved to be useful materials in polymer composites, where transfer of mechanical loads is necessary. Addition of carbon fibres to polysulfone produces composite materials which are used to manufacture different medical devices such as plates, screws, joint replacements and bone implants [1, 2, 12, 13].

Designing of such biomaterial composites is mostly oriented towards obtaining high-strength materials whose Young's modulus matches that of the replaced tissue, though it is not the only goal. By using well-known polymers reinforced with carbon fibres differing in type and form, it is possible to obtain implantable materials with controlled Young's modulus, strength and deformability fitted to surrounding tissue. This is an important factor in the treatment of all types of tissue.

Carbon composites applied in the tissue treatment exhibit a number of advantageous properties, such as transparency to X-rays, the possibility to obtain complex shapes as well as reduced implant sizes. Moreover, the polymer composite materials are fully compatible with modern diagnostic methods such as computer aided tomography (CAT) and magnetic resonance imaging (MRI) as they are not magnetic [13]. All these features allow these materials to often replace metallic implants.

* AGH – University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Biomaterials
** Jagiellonian University Collegium Medicum, Department of Immunology

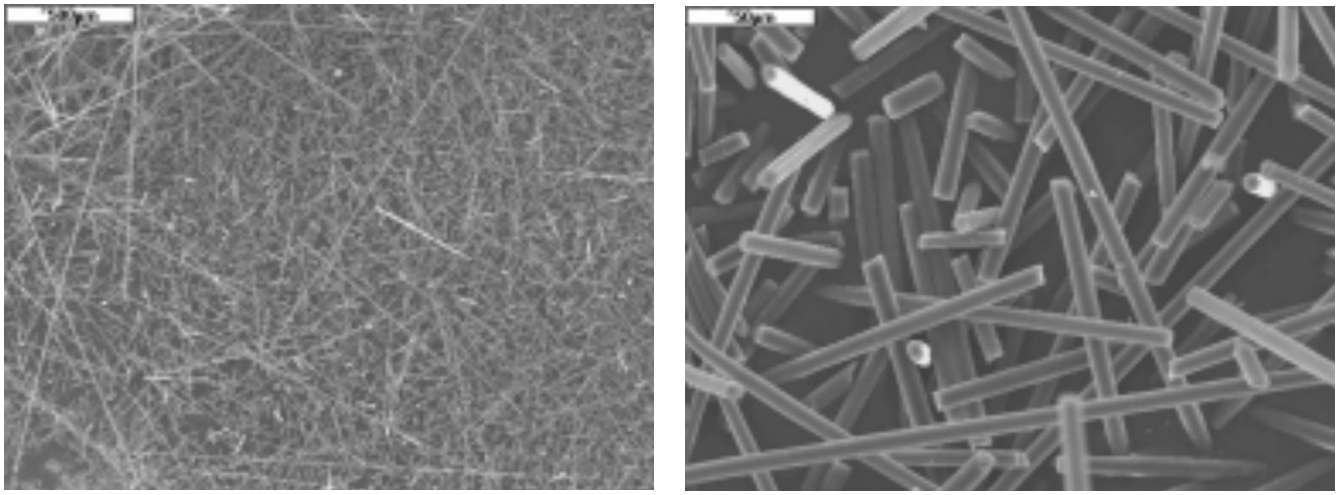

Figure 1. SEM micrographs of initial and fragmented carbon fibres, 50× and 500× magn.

The PTFE/PVDF/PP copolymer contains tetrafluoroethylene, vinylidene fluoride as well as propylene units. It is an interesting material, which can be applied as a biocompatible carbon fibres composite. The PTFE/PVDF/PP copolymer possesses a low mechanical strength (0.5-0.8 MPa) and high elongation at break (ca. 100%).

Polymers, such as PSU and the PTFE/PVDF/PP copolymer, mixed with carbon fibres (depending on the fibre content and distribution in the polymer matrix) to obtain composites should be an ideal material for structural implants, whose mechanical properties are close to that of native tissue. However, dispersion of carbon fibres in the polymer matrix influences not only mechanical properties, but also microstructure, magnetic and electric properties of the materials. Another important property of composite materials is the possibility to modify their microstructure and surface properties due to the introduction of carbon fibres in polymer matrix varying in amount and the kinds of surface functional groups present. Such features of polymer composite materials influence their biological properties.

The aim of the work was to study biocompatibility of two kinds of polymers and their composites with short carbon fibres under *in vitro* conditions.

Methods

Polysulfone ("PSU", M_w 26 000, cat. no 37,429-6) and poly (tetrafluoroethylene-co-vinylidene fluoride-co-propylene) ("TFL", cat. no 45, 458-3) were purchased from Aldrich Chemical Comp. Inc. Milwaukee, USA. Dichloromethane and acetone (POCH S.A., Gliwice, Poland) were used to prepare polymer solutions. Polymer samples, namely: K_{PSU} (with polysulfone matrix) and K_{TFL} (with poly (tetrafluoroethylene-co-vinylidene fluoride-co-propylene matrix) with fragmented FT-300 carbon fibres (no sizing, Soficar, France) were prepared by the casting technique. Pure cast polymers were the reference samples. The materials were prepared in the form of discs, 20 mm in diameter. The discs were UV sterilized. The cells used for assessment of the materials were hFOB-1.19-line human osteoblasts and HS-5-line human fibroblasts (ATCC, University Boulevard, Manassas, Canada) placed on the smooth surface of the samples. The level of type I collagen produced from both types of cells was determined by ELISA. The viability of fibroblasts and osteoblasts was assayed through MTT.

Results

SEM micrographs of initial and fragmented carbon fibres are shown in Fig. 1. Schematic drawings of samples of pure polymers and composites with short carbon fibres are displayed in Fig 2.

Results of *in vitro* studies are presented in Figs. 3, 4, 5 and 6. The bars represent percentage values of fibroblast and osteoblast viability and the level of collagen produced by these cells, assuming 100% survival for control cells. Acceptable test error is 5%.

Discussion

The highest viability of fibroblasts was observed on the surface of pure polysulfone (Fig. 3). The corresponding carbon fibre composite (K_{PSU}) exhibited a viability comparable to the samples of TFL. The PSU samples were found to have the highest level of collagen produced by these cells (Fig. 4).

Investigation of TFL composite samples (K_{TFL}) showed that the level of collagen produced by fibroblasts on their surface is higher than on the surface of reference samples (Fig. 4). Furthermore, despite the

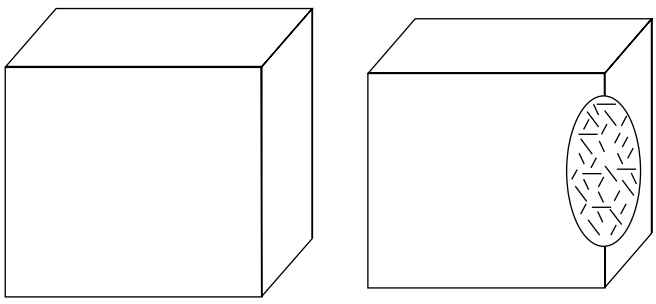

Figure 2.

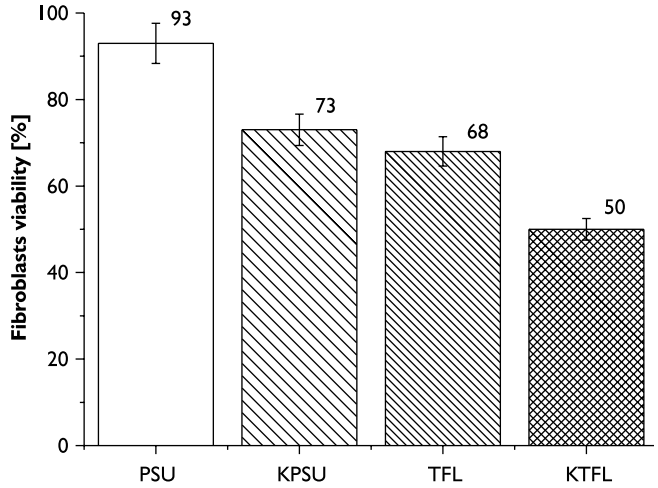

Figure 3. Viability of fibroblasts on the surfaces of the examined materials.

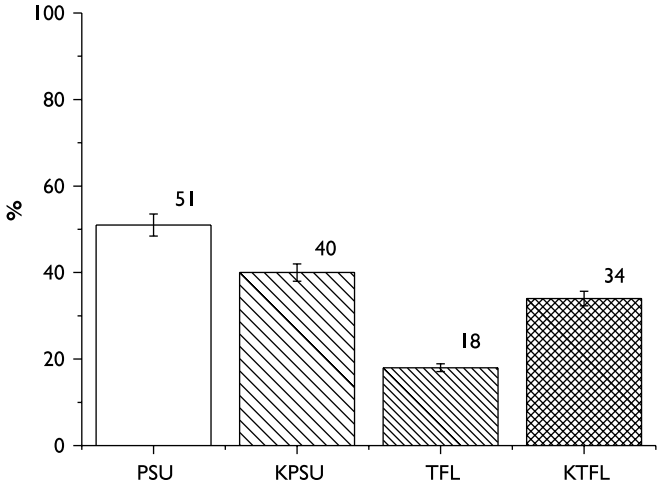

Figure 4. Levels of collagen (%) produced by fibroblasts on the examined materials surfaces, normalized to the level of the control.

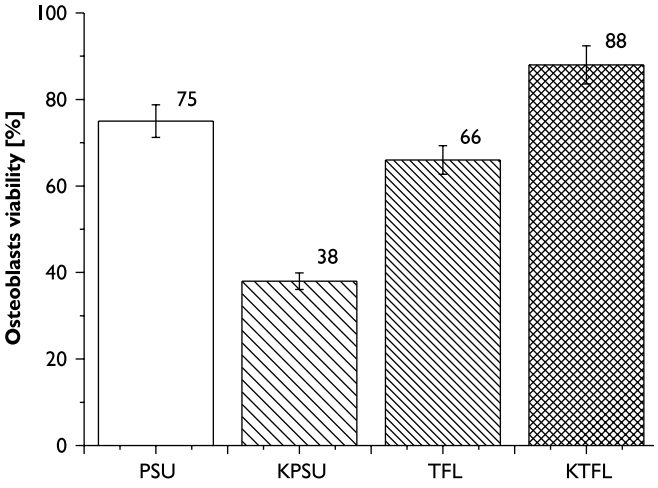

Figure 5. Viability of osteoblasts on the surfaces of the examined materials.

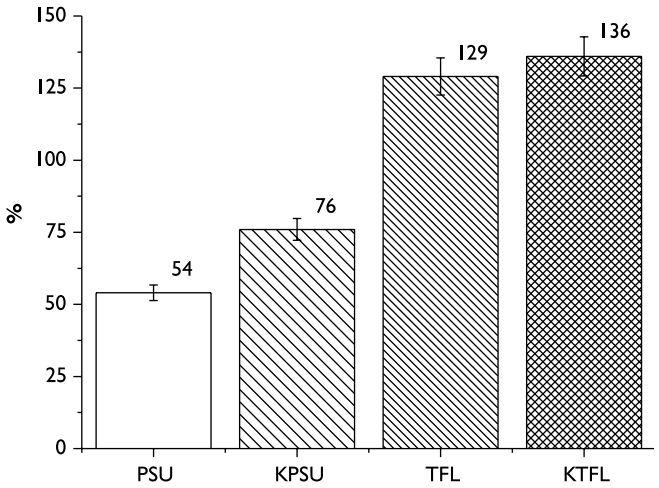

Figure 6. Levels of collagen (%) produced by osteoblasts on the examined material surfaces normalized to the level of the control.

lower fibroblast viability, the cells interacting with the composite samples produce more collagen (Fig. 3).

Osteoblast viability (Fig. 5) is the highest in contact with the K_{TFL} surface. Viability of osteoblasts on K_{PSU} is about half of the reference sample (Fig. 5), but the level of collagen produced on the composite is significantly higher than on pure PSU (Fig. 6). This shows the favourable influence of carbon fibres on the biocompatibility of the composite materials with osteoblasts.

The results of biological investigations exhibit differences in cell response depending on the type of polymer. Both fibroblast and osteoblast viability differs with the type of material. This could be understood as different biocompatibility of the investigated materials with respect to different cells. Results presented in the work indicate influence of composite materials on osteoblasts.

The differences in viability and level of collagen produced by the cells likely results from the different surface energies of the investigated samples. The surface energy of carbon-polymer composites is different from pure polymers due to reaction of carbon fibre surfaces with polymer functional groups. The results indicate that osteoblasts, unlike fibroblasts, are sensitive to the level of biomaterial surface energy.

Address reprint requests to: Marta Błażewicz, AGH – University of Science and Technology, Faculty of Materials Science and Ceramics, Department of Biomaterials, Al. Mickiewicza 30, 30-059 Krakow, Poland
Phone: (+48-12) 617 25 09
E-mail: mblazew@uci.agh.edu.pl

References

1. Teoh SH, Tang ZG, Hastings GW: Thermoplastic polymers in biomedical applications – structures, properties and processing; in Black J, Hastings G, (Ed): Handbook of biomaterial properties. London, Chapman & Hall, 1998.
2. Min Wang: Developing bioactive composite materials for tissue replacement. Biomaterials 2003, 24: 2133
3. Nishimura T: Polymer materials for blood purification; in Tsuruta T, Hyashi T, Kataoka K, Ishihara K, Kimura Y, (Ed): Biomedical application of polymeric materials. FL, CRC Press, Boca Raton, 1993, p 191
4. Zhao CS, Liu T, Lu ZP, Chen LP, Huang J: Evaluation of polyethersulfone hollow fiber plasma separator by animal experiments. Artif Organs 2001, 25: 60
5. Changsheng Zhao, Xiangdong Liu, Motoyoshi Nomizu, Norio Nishi: Blood compatible aspects of DNA-modified polysulfone membrane-protein adsorption and platelet adhesion. Biomaterials 2003, 24: 3747
6. Urban E, King MW, Guidoin R: Why make monofilament sutures out of polyvinylidene fluoride?. ASAIO J 1994, 40: 145
7. Klinge U, Klosterhalfen B, Ottinger AP, Junge K, Schumpelick V: PVDF as a new polymer for the construction of surgical meshes. Biomaterials 2002, 23: 3487
8. Marois Y, Guidoin R, King MW, Martin L, How T, Douville Y: Polyvinylidene fluoride (PVDF) as a biomaterial: from polymeric raw material to monofilament vascular suture. J. Biomed. Mater. Res. 1995, 29: 1525
9. Mary C, Marois Y, King MW, Laroche G, Douville Y, Martin L, Guidoin R: Comparison of the *in vivo* behavior of polyvinylidene fluoride and propylene sutures used in vascular surgery. ASAIO J 1998, 44: 199
10. Pampuch R, Błażewicz S, Chłopek J, Powroźnik A, Wajler C, Pamuła E: Włókniste i kompozytowe materiały węglowe. Inżynieria Materiałowa 1993, 5: 116
11. Błażewicz M: Węgiel jako biomateriał. Prace komisji Ceramicznej PAN. Ceramika 63, 2001
12. Ratner BD, Hoffman AS, Schoen FJ, Lemons JE (Ed): Biomaterials science: an introduction to materials in medicine. San Diego, Academic Press, 1996
13. Ramakrishna S, Mayer J, Wintermantel E, Kam WL: Biomedical applications of polymer-composite materials: a review. Composites Science and Technology 2001, 61: 1189