PROTEINS ADSORPTION ON BIOMEDICAL CARBONS

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Introduction

Recent research into biocompatibility has identified the important role that surface properties of biomaterials (chemical nature, topography, microstructure) and processes of proteins adsorption play in cellular interactions [1-2].

Collagen and fibrinogen are the proteins of particular interest. Collagen is the most abundant structural protein of the extra-cellular matrix and is involved in many important biological functions such as tissue structuring and biorecognition process [3]. Fibrinogen is one plasma protein that has received much attention due to its crucial role in blood clot formation [4] .

Many types of carbon materials have been used in medicine as long- or short-term implants or as biosensors. Low-temperature isotropic carbon (LTI), a reputed blood-compatible material, is widely used in artificial heart valve construction, while glass-like carbon (GC) in the replacement of small joints and in dentistry [5].

The aim of this study is to examine the adsorption of collagen and fibrinogen on model carbons (LTI and GC) in order to get insight into the relation between surface properties and protein adsorption process. Three techniques are used to study the surface of carbon substrates before and after protein adsorption: X-ray photoelectron spectroscopy (XPS) to determine surface composition, water contact angle to assess hydrophobicity and atomic force microscopy (AFM) to examine topography.

Experimental

Substrata

Glass-like carbon (GC) was obtained by carbonisation process of phenol-formaldehyde resin. Low-temperature isotropic carbon (LTI) was deposited on fused quartz from methane.

Adsorption study

Adsorption of type I collagen and fibrinogen were made as follows: I) protein solution (concentration 40μg/ml) was brought in contact with the substrates for 2h at 37°C, II) after incubation the aqueous phase was replaced by water and this was repeated by washing the sample without air exposure, III) the samples were analysed both in wet and in dried state.

Methods

The samples before protein adsorption were characterised by XPS (SSI X-Probe, Surface Science Instruments), water contact angle (sessile drop method) and AFM (Nanoscope III, Digital Instrument, Santa Barbara, CA). After adsorption the protein film was examined: I)in situ under water, by AFM, to study topography, II) after drying in nitrogen flow, by XPS, to asses amount adsorbed and III) by sessile drop method to provide complementary information about the adsorbed film.

Results and discussion

The surface characteristics of the carbons used for adsorption of proteins and their representative AFM topographic images are shown in TABLE 1 and FIGURE 1a, b, respectively. On the surface of GC the concentration of oxygen is much higher than on LTI. In addition small amount of nitrogen, originating from precursor resin is present. Elemental analysis of both carbons is consistent with their water contact angles: lower value of θ for GC, revealing lower hydrophobicity, goes along with higher oxygen concentration.

	Elemental composition, mole fraction[a]			θ water contact angle[b]	R_{rms} roughness[c]
	C	O	N	degree	nm
GC	91.5	7.4	1.0	85±2	2
LTI	98.7	1.3	-	103±3	5

[a] Determined by XPS
[b] Determined by sessile drop method; mean value of 10 measurements and confidence interval at 0.95
[c] Determined by AFM; on 5x5μm area, expressed as the standard deviation of the topographical height

TABLE 1. Surface properties of carbons

On the other hand, the surface Rrms roughness of LTI is higher than GC. The AFM images show that GC surface is quite smooth with some regular relief probably resulting from the grooves in the mould used in production process, whereas on the LTI surface grain-like features are observed.

	N/C	N/C after adsorption of		θ water contact angle after adsorption of (degree)	
		collagen	fibrinogen	collagen	fibrinogen
GC	0.01	0.12	0.20	70±2.7	74±2.8
LTI	-	0.14	0.10	72±1.7	80±1

[a] Determined by XPS
[b] Determined by sessile drop method; mean value of at least 10 measurements and confidence interval at 0.95

TABLE 2. N/C and water contact angles of carbon substrata before and after protein adsorption.

XPS analysis after adsorption process shows an increase of N/C ratio on the surface of both substrata and a decrease in hydrophobicity [TABLE 2]. Note that nitrogen is due to adsorbed proteins, thus N/C ratio is a parameter used to *estimate adsorbed amount of proteins.*

N/C and θ of both carbon substrata with adsorbed collagen are similar suggesting that neither initial hydrophobicity nor topography influence adsorption of collagen. The adsorbed collagen film is clearly visible on the surface of GC as a mesh of elongated fibrous features [FIG.1c]. On the other hand, any morphological changes are seen after collagen adsorption on LTI [FIG.2d]. This phenomenon is due to too high roughness of the carbon substrate, comparable with the size of collagen molecule [3].

N/C value of GC sample with adsorbed fibrinogen, twice

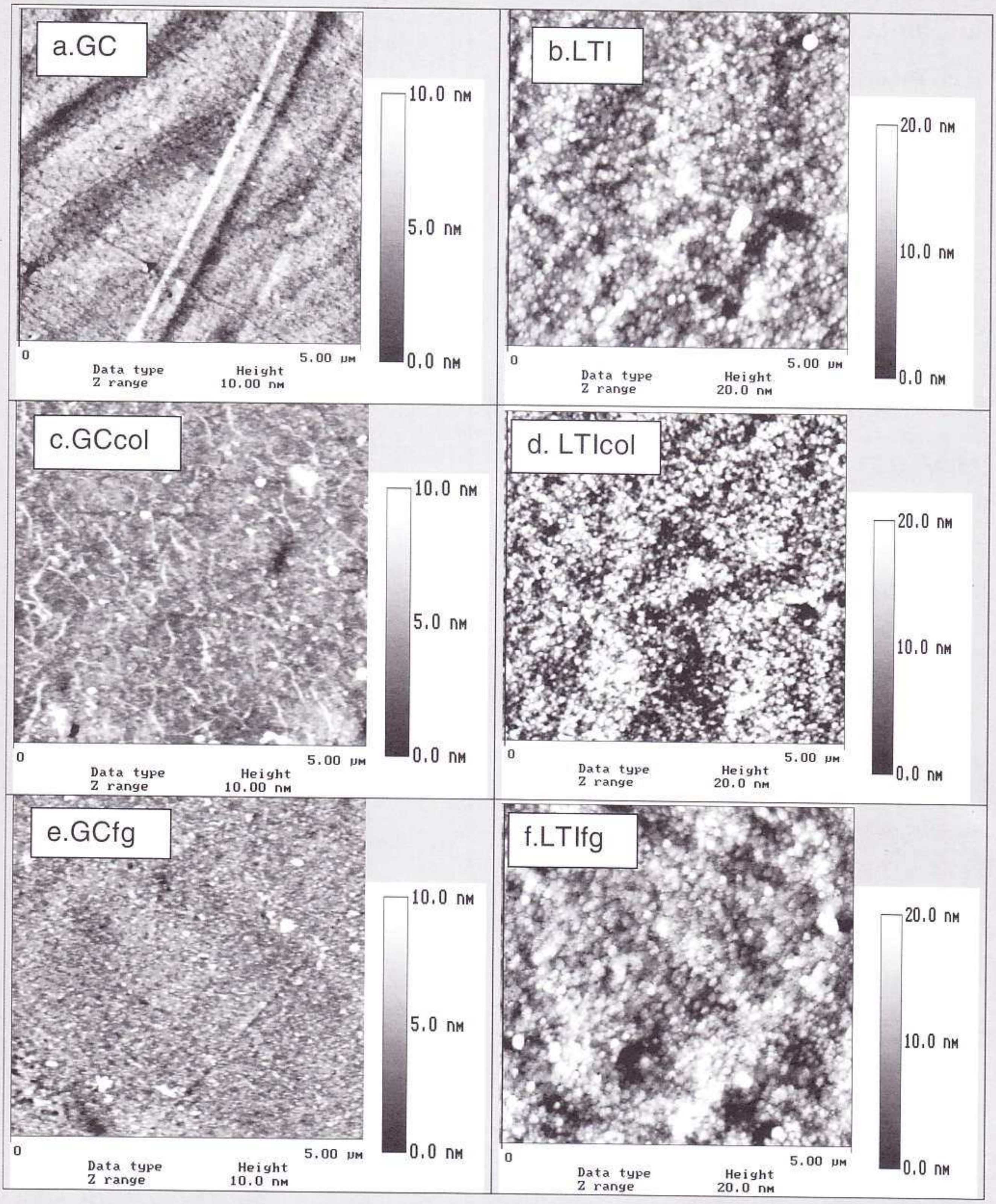

FIG.1. AFM topographic images recorded under water for GC (z-range: 10nm) and LTI (z-range: 20nm) before and after adsorption of collagen and fibrinogen.

higher than that of LTI [Table 2], suggests that lower initial hydrophobicity and/or lower roughness promotes adsorption phenomena. Adsorbed film on GC is smooth, homogeneous and continuous [FIG.1e], while any changes of LTI topography are visible after adsorption [FIG.1f] due to high substrate roughness, comparable to the size of fibrinogen (the same reason as in the case of collagen).

In conclusion we can say that processes of protein adsorption depend on the type of protein and properties of the substrate (chemical nature, topography). Surface properties of carbon substrata seem do not influence adsorption of collagen while they look to be important in the case of fibrinogen.

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References

[1] Schakenraad JM, Busscher HJ: Cell-polymer interactions: the influence of protein adsorption. Colloids and Surfaces 1989;42:331-343.
[2] Tamada Y, Ikada Y: Effect of preadsorbed proteins on cell adhesion to polymer surfaces. Journal Colloid Interface Sci 1993;155:334-339.
[3] Kadler, K.E.; Holmes, D.F.; Trotter, J.A.; Chapman, J.A. Collagen fibril formation. Biochem J. 1996, 316,1-11.
[4] Arai T, Norde W. The behaviour of some model proteins at solid-liquid interfaces. 1. Adsorption from single protein solutions. Colloids and Surfaces 1990;5:1-15.
[5] Feng L, Andrade LD. Protein adsorption on low-temperature isotropic carbon: II. Effects of surface charge of solids. J Colloid Interface Sci 1994;166:419-426.

IN VIVO BIOCOMPATIBILITY OF CARBON FIBERS /PSU COMPOSITE

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In laryngology for treatment of tissue of larynx resulting from cancer or injuries synthetic materials are becoming frequently used. In the decade there have been made trials with allogenic materials application in larynx and trachea reconstruction such as silastic, teflon and bioglass. Reconstruction of the loss larynx tissue requires recreation of natural anatomic conditions. This is possible when the properties of an implant material are similar to cartilage tissue i.e. it preserves the appropriate shape end elasticity,and its microstructure enablesconnective tissue of larynx to penetrate into micropores of the implant.
Much work have been done on the materials used for attificial tracheas, but a precise machanical evaluation of these structures has not yet been performed.
The present study examined biocompatibility of two types of composite materials which have different mechanical properties. We determined the mechanical properties of implant materials and compared them with native larynx.
Composite materials have been prepared using polysulfone and two type carbon fibers differing in their form (carbon tissue, carbon unwoven fabric) .Two types of materials were prepared; unwoven fabric / PSU - K_w, unwoven fabric / carbon tissue / PSU - K_{wt}. The results of tensile strength and Youngs modulus of two kind of materials exhibits that K_w composite has lower strengh and modulus compare to K_{wt} materials.
The composite implants has been used to reconstruction of experymentaly prepared defects in the thyroid cartilage of the sheep. The tissue samples removed from the implant site together with adjoining tissue were subjected to routine histological analysis. Tissue section were stained with hematoxylin and eosin (H&E). A morphological description of the tissue surrounding and growing into the implants was made.

The nature of interaction between the biological environment and composite implants is clerly influenced by type of implants. The material denoted as K_w having lower Youngs modulus leads to a faster and more intesity tissue response, which simultaneously can influence regenration and repair time of larynx tissue. The histological inspection has shown the formation of connective tissue capsule with numerous fibrocytes and collagen fibers filling the defect.
This study showed that biological behaviour of composite implants may depend not only their chemical state but also on mechanical properties of biomaterials.

References

[1] Błażewicz M., Błażewicz S., Konieczna B., Pamuła E., Nowy materiał dla laryngologii Inżynieria Biomateriałów 2001. 77 - 79.
[2] Błażewicz S., Pamuła E., Maliński M., Pilch J., Bielecki I., Hybrid composite implants in laryngology Prace Mineralogiczne 2000, 89, 19 - 25.
[3] Flint P., Corio R.,Cummings C., Comparison of soft tissue response in rabit following laryngeal implantation with hydroxylapatitue, silicone rubber and teflon, Ann-Otol-Rhinol-Laryngol. 5, 106, (1997), 399-407
[4] Righi P., Wilson R., Gluckman J., Thyroplasty using a silicone elastomer implant. Otolaryngol. Clin. North. Am. (1995), 28, 2, 309-316