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### RESEARCH ARTICLE

#### IN-VITRO IMMUNOCOMPATIBILITY & CHARACTERIZATION AT NANOSCALE OF THIN FILMS AND VASCULAR MEDICAL DEVICES.

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#### Abstract

Immunocompatibility comprises a complex response which depends both on the physico-chemical characteristics of the biomaterial and on the hereditary and acquired ability of the recipient to react.

**Objective:** The objective of the present work was the comparative study of the immunocompatibility of different materials and their morphological characterization.

**Methods:** The three types of thin films used in this work were the titanium nitride [TiN], Titanium [Ti] and the amorphous hydrogenated diamond-like carbon [a-C:H], which are deposited onto a silicon substrate by Magnetron Sputtering and Plasma-Enhanced Chemical Vapor Deposition (PECVD), respectively. Medical devices used in this study, were the silicon coated Latex irrigation catheter and the endoprosthesis such as vascular grafts [Dacron, PTFE] and stent grafts [Nitinol stent graft, Cobalt Chromium stent graft]. Bare silicon substrate and serum were used as a “negative” control and the Latex [elastomer-elastic hydrocarbon polymer] as a “positive” control. Complement C5 convertase [C5c] activation was assessed using the sandwich enzyme immunoassay-sandwich ELISA for the specific time periods [0min, 15 min, 30 min and 60 min] at wavelength of 450nm. The morphological characterization of the materials was conducted by Scanning Electron Microscopy (SEM). The study of biomaterials topography was conducted by Atomic Force Microscopy (AFM) and their surface wettability properties by Contact Angle measurements.

**Results:** Our results show that both groups of materials (the nanomaterials and the medical devices) are not likely to cause any immunological adverse reaction and as a result might be characterized and selected as excellent candidates for medical applications.

**Conclusions:** The effectiveness of the study is attributed to the measurement of a single factor in serum in order to acquire important information about the materials’ properties e.g immunological response. The possibility to modify the above tested parameters during

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material synthesis and manufacture could allow limiting their immunological response and improving their immunocompatibility.

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## **Introduction:-**

Complement activation has been recognized as an important component of the biocompatibility of materials. When a biomaterial comes into contact with blood, complement activation is one of the reactions of the inflammatory response. Complement plays an important role in the body's defence mechanism and has many clinical consequences. The complement system is a part of the innate and acquired immune systems that help fight infections. Activation of complement occurs via three pathways: the classical, alternative and lectin pathways[1-3]. C5 convertases are serine proteases that cleave both C3 and C5 component of complement. Alternative pathway C3/C5 convertases formed with monomeric C3b (C3b, Bb) because of their weak interaction with C5 primarily cleave C3 thereby opsonizing the cell surface with C3b. In contrast, C3/C5 convertases formed with a high density of C3b/cell exhibit higher affinities for C5 below the physiological concentration of C5 in blood. These C3/C5 convertases bind C5 efficiently and cleave it at a velocity approaching  $V_{max}$  thereby switching the enzyme from C3 cleavage to production of the cytolytic C5b-9 complex. Studies of the structure of C3/C5 convertases have postulated that C4b-C3b and C3b-C3b dimmers form high affinity C5 binding sites while studies have shown two binding sites in C5 for the convertase in addition to the C5 cleavage site. Together, these studies indicate that with increasing deposition of C3b on the surface, C3b complexes are formed which through multivalent attachment bind the substrate C5 with higher affinities, thereby converting the low affinity C3/C5 convertases to high affinity C5c [4]. On the other hand from a nanomaterial's point of view, understanding the molecular basis of its properties in complement activation could provide approaches to ideal material design and nanoengineering strategies for eliminating acute allergic responses to future nanomaterials for medical applications [5]. In this study a sandwich ELISA (Enzyme Linked Immunosorbent Assay) for C5c complement activation induced by biomaterials is validated. In vitro evaluation was performed with human serum. ELISA kit is commercially available to measure the complement protein C5c. A screening test to evaluate C5c complement activation of candidate biomaterials using this kit involved comparing its capacity to activate complement with that of reference biomaterials. Complement activation of the reference biomaterials was investigated and the complement protein being assayed was C5c (C5 convertase).

## **Materials and Methods:-**

### 2.1 Materials

The biomaterials used in the experiment are divided into 3 categories. A) Thin films: Titanium Thin Film [Ti], Titanium Nitride Thin Film [TiN], Amorphous hydrogenated diamond like carbon [a:C-H] and Silicon substrate [Si] was obtained from LTFN (Laboratory for Thin Films Nanosystems and Nanometrology, Physics Department - Aristotle University Thessaloniki). B) Medical devices: Silicon coated irrigation latex catheter [Foley] and Latex was obtained from General Hospital of Thessaloniki "St.Dimitrios" and C) Endografts: Polytetrafluoroethylen [PTFE], Polyethyleneterephthalate [Dacron] and Nitinol stent graft was obtained from W. L. Gore & Associates, Inc. Powerlink stent graft was obtained from Endologix, Inc.-Irvine, California. Latex [elastomer-elastic hydrocarbon polymer] was used as a positive control and bare silicon substrate and human serum was used as a negative control.

### 2.2 Pooled serum

For pooled serum, blood was drawn from healthy blood donors into Vacutainer sterile tubes, allowed to coagulate at room temperature for 120 min and centrifuged at 1000x g for 20 min. The serum fractions were pooled and kept at -70°C until further analysis.

### 2.3 Sterilization and Incubation

Ethylene oxide terminal sterilization is used to render the biomaterials within the final sterile barrier package free from viable microorganisms. Biomaterials were cut into 0, 5 cm<sup>2</sup> and 1 cm<sup>2</sup> pieces. The samples were immersed and incubated in serum at different time intervals [0 min, 15 min, 30 min, 60 min] at room temperature, with 0,5 ml or 1 ml of normal human serum. Human serum samples require about 50 times dilution. Suggested dilution is 10 ML sample in 490mL PBS [50-fold dilution]. The sample should be dissolved in 0.02mol / L PBS (pH = 7.0-7,2). The Wash Buffer is stable for 5 days when stored at room temperature (20 to 25 °C).

#### 2.4 Analysis of C5c activation in human serum by sandwich ELISA

Determination of concentrations of C5c of complement was performed by ELISA Kit for Human Complement C5 Convertase, Soluble (C5c) enzyme immunoassay (Uscn Life Science Inc. Wuhan). The principle of the procedure for the C5c complement component assayed was a three-step procedure utilizing: (1) a microassay plate pre-coated with an monoclonal antibody specific to C5c which binds specifically to the C5 complement component, (2) a horseradish peroxidase conjugated antibody to antigens of the component, (3) a chromogenic substrate. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of  $450 \text{ nm} \pm 2 \text{ nm}$ . The concentration C5c in the samples is then determined by comparing the O.D. of samples to the standard curve.

#### 2.5 Morphological evaluation by Scanning Electron Microscopy (SEM)

Information about the samples surface topography and composition were taken by technique of Scanning Electron Microscopy (SEM). Sample preparation is done with standard stabilization protocol of the samples with glutaraldehyde and then by drying of the samples. Drying of the samples was made in three cycles with the addition of aqueous solutions of ethanol concentration 70%, 90% and 100% v/v sequentially. Each solution was left at samples about 30 minutes. After removing the last ethanol solution, samples were allowed to dry at room conditions in a laminar flow cabinet on air atmosphere.

#### 2.6 Topographical evaluation by Atomic Force Microscopy (AFM)

The study of topological properties of samples surface in this experiment was performed with atomic force microscopy (AFM). The software used to estimate the values of the average roughness and peak-to-valley is the AFM model Ntegra, SPM, NTMDT. The samples were thin films [Ti], [TiN], [a:C-H] and [Si].

#### 2.7 Wetting behavior of samples by Contact angle measurement (CA)

Determination of hydrophilicity (hydrophobicity) of materials and their interactions with body fluids (serum) was performed by Contact angle measurement (CA). In experiment, the drop of water used for contact angle measurement, had a volume 5mL and the free surface energy of water was equal to 72,8 mN/m. The contact angle measurement of the samples was static and performed with goniometer (KSV Instruments LTD, CAM200). Determination of the free surface energy with the drop of deionized water was performed with the help of the Solid Free Surface Energy Calculation program. The contact angle measurements were made by testing the following solutions: deionized water, PBS, DMEM DMEM10 (DMEM + 10% FBS + 1% A / B). These solutions were used to study the behavior of materials in the various solutions that were in contact.

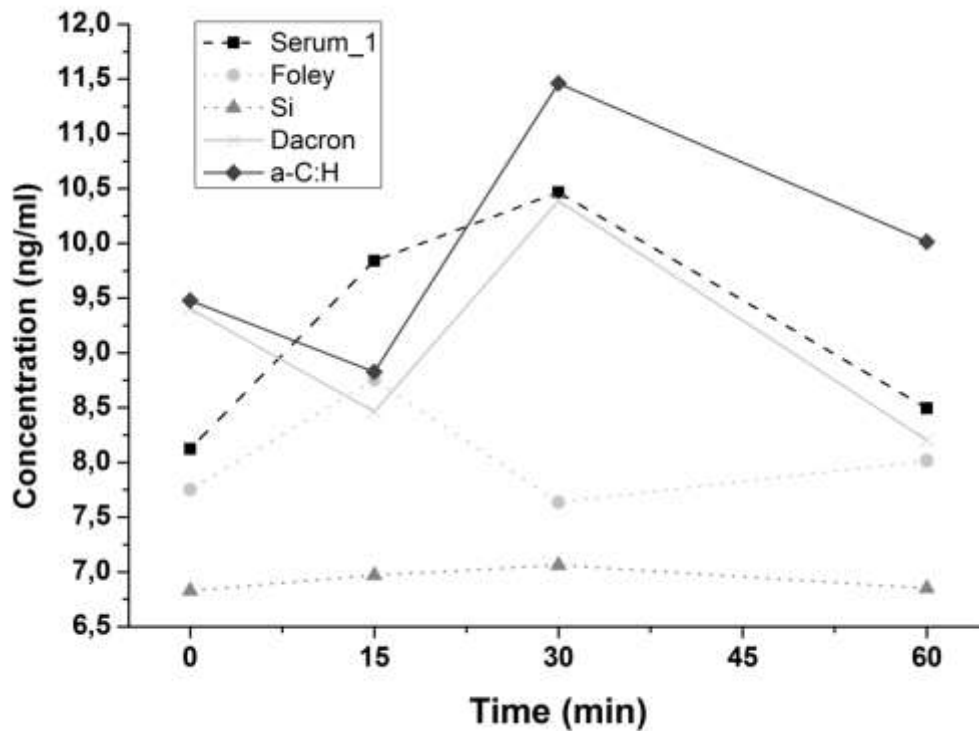
### Results:-

#### 3.1 C5c activation by sandwich ELISA

All the biomaterials were found to be activators of complement but to different degrees. It is shown that substrate conversion, expressed as OD at 450 nm, increases when the biomaterial is for a longer time in contact with plasma, indicating more C5-converatse formation. Since in this experiment after an incubation time of 30 min there was sufficient conversion, this incubation time was chosen in further experiment as a standard time.

#### First group of materials

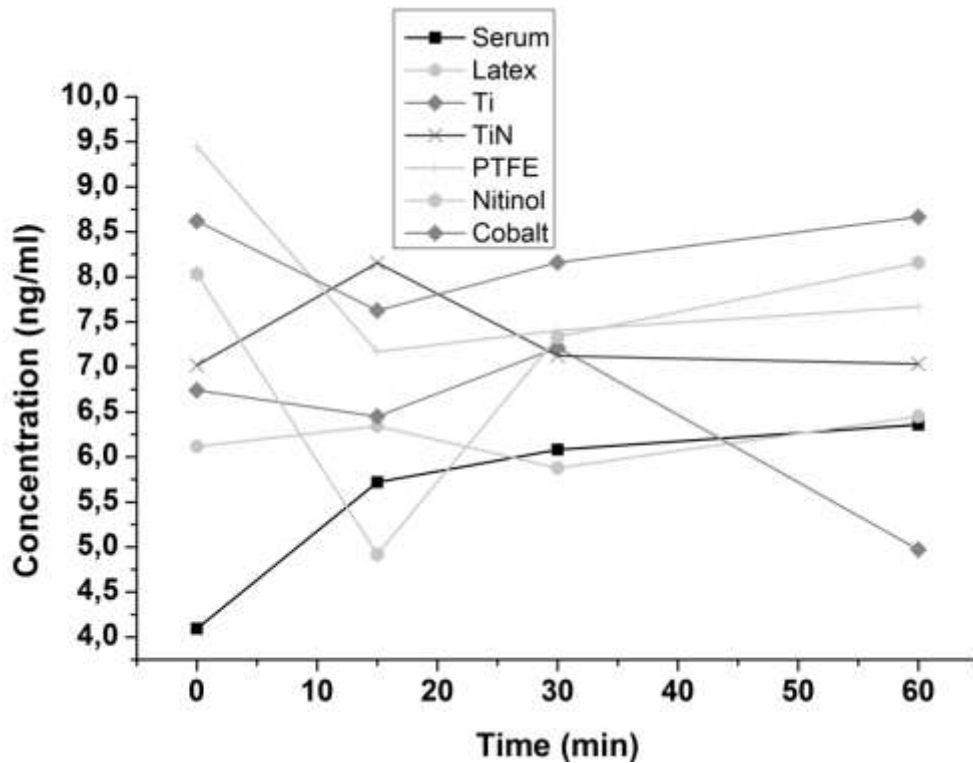
In the first group of materials regarding the concentration of activated C5c, the ranking of the biomaterials was a-C:H > Dacron > Serum\_1 > Foley > Si. Starting at 15 min, C5c was detectable in the serum samples but no significant difference appeared among the biomaterials before 30 min, except the Si. After 30 min a significant increase of C5c was observed for Dacron, a-C:H thin film and for the Serum\_1 negative control. At 1 h, a-C:H was significantly different from Foley, Dacron, Serum\_1 and Si. Control ran with bare silicon substrate showed that the peace of silicon substrate was not inducing complement activation to a level that would interfere with the complement activation mediated by the biomaterials tested.



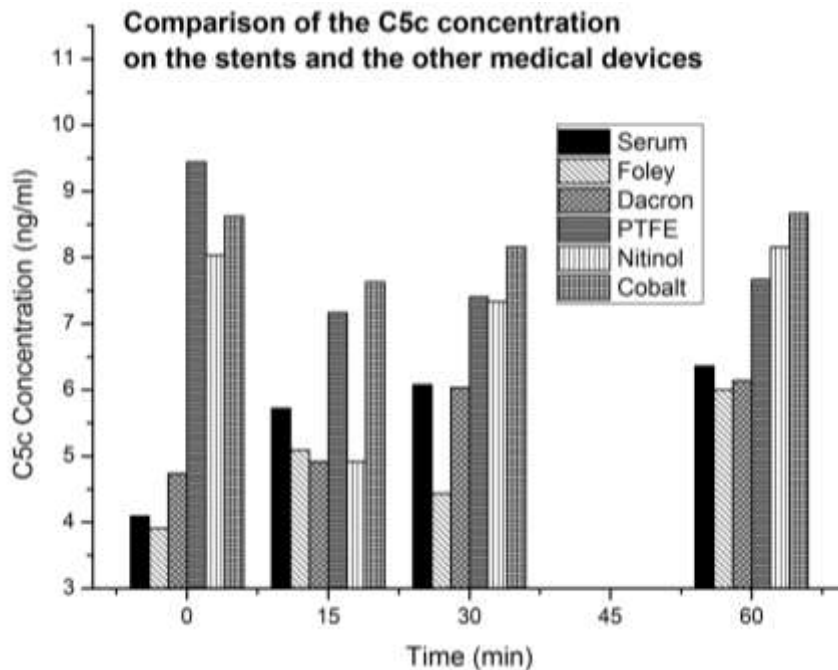
**Figure 1:-** Quantification of C5c in human serum after incubation of the first group of materials. Discs of materials were incubated with human plasma for the specific time periods (0 min,15 min,30 min,60 min).Serum was assayed for C5c activation using sandwich ELISA at wavelength of 450 nm.

#### Second group of materials

In the second group of materials the ranking of biomaterials was: Cobalt chromium > Nitinol > PTFE > TiN > Serum\_2 > Latex > Ti. During the first 15 minutes of incubation a significant decrease of C5c concentration was observed for PTFE, Cobalt chromium, Nitinol and Ti thin film. At 15 min of incubation, C5c was detectable in the serum samples and difference appeared among the TiN metallic thin film and the other biomaterials of the second group. After 30 min formation of C5c started to level off for Cobalt chromium, PTFE, TiN and Nitinol, while for Ti metallic thin film, from 30 to 60 min, a constant decrease could be observed. After 1 h, a plateau was reached for Cobalt chromium, PTFE, Nitinol and TiN, while C5c for Ti thin film continued its decrease. Control ran with Latex showed that the elastomer was not inducing complement activation to a level that would interfere with the complement activation mediated by the biomaterials tested.



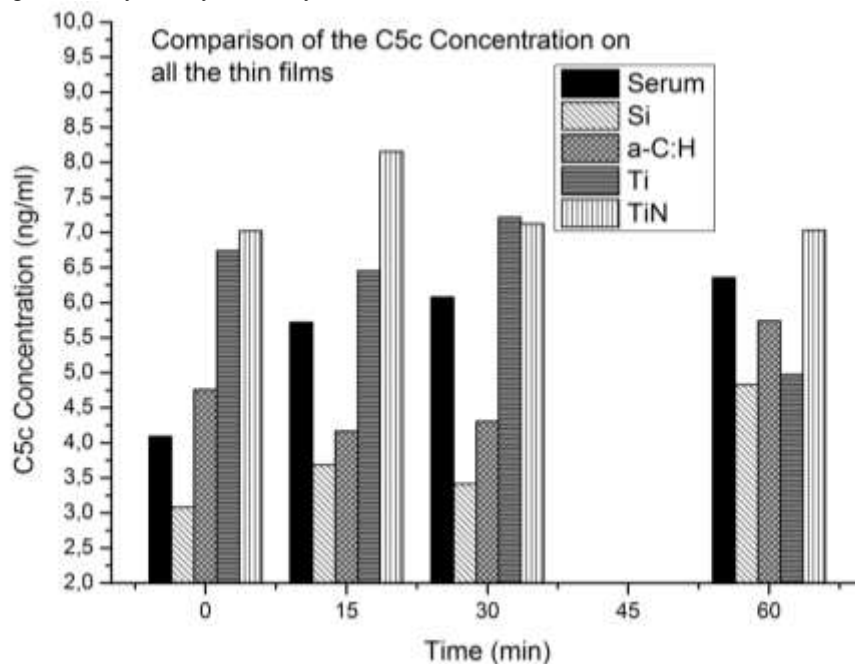
**Figure 2:-** Quantification of C5c in human serum after incubation of the second group of materials. Samples were incubated with human plasma for the specific time periods (0 min,15 min,30 min,60 min). Serum was assayed for C5c activation using sandwich ELISA at wavelength of 450 nm.



**Figure 3:-** Comparison of C5c activation of thin films (first & second group).

As shown in Fig. 4 the material with greater activation of C5c factor, is a metallic thin film a-C:H. The same kinetic curve of C5c factor activation was observed for the sample of biomaterial Dacron. Human serum was used as a negative control and as an incubation medium. The results showed that initially the C5c is already activated; a

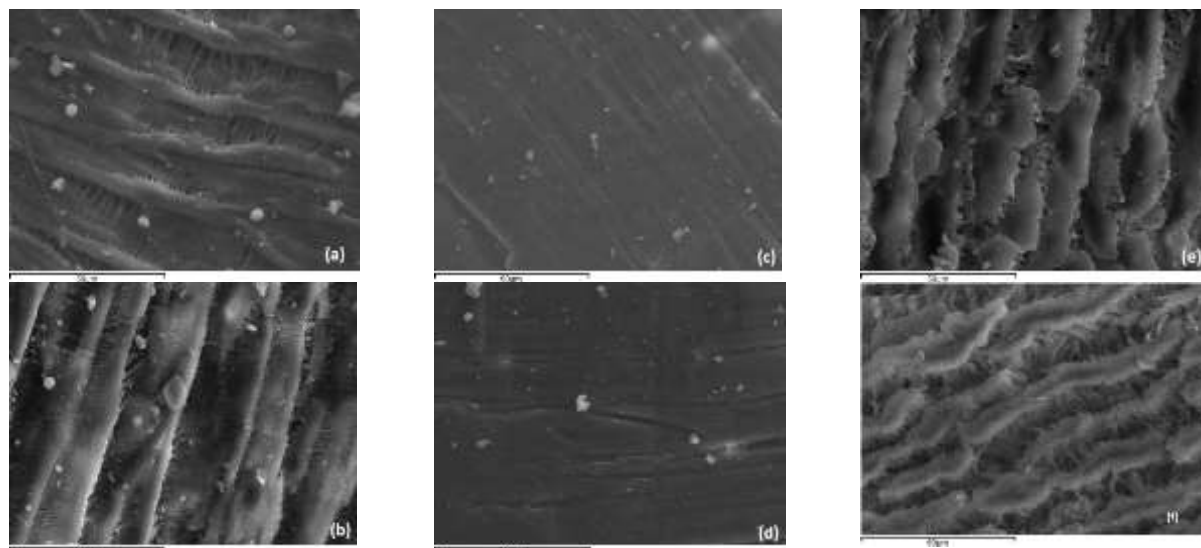
situation that warrants from handling during the preparation of the sample, such as blood sampling, sample preparation, experimental procedure, conditions which provides proved complement activation to a certain extent. Serum\_1 and Serum\_2 which was used as a negative control and incubation agent, shows a great difference in concentration of activated C5c factor. These samples were from different phases of the experiment and the result was attributed to different operating conditions of the first and second phase of experiment and to the optimizing of the performance of experiment in the second phase. The Si, which was used as a negative control in this experiment shows that it causes activation of C5c to degree, is almost negligible without or with very little change during all incubation times. The result was expected as the silicon is proven as a bio-inert and bio-resistant material. The TiN and Ti metal thin films show the results approximately at the same level as a Si. Dacron demonstrated the highest activation of C5c factor. Dacron has the same kinetic curve of C5c factor activation with the a-C:H metallic thin film. Approximately at the same level is the PTFE vascular graft following by the Powerlink stent graft and Nitinol stent graft. The Foley is approximately at the same levels of activation with metal thin films TiN and Ti. From the group of medical devices, the material with the lower activation of C5c is the Latex (elastomer). The results were not expected, but not surprising since the Latex is not proposed negative control according to the EN ISO 10993-12:2004 for blood and immune compatibility. It was selected because of his extensively applications in medicine and known allergen activity and cytotoxicity.



**Figure 4:-** Comparison of C5c activation of medical devices (first & second group).

### 3.2 Surface morphology by Scanning Electron Microscopy

At this scale there is no significant difference of SEM images of medical devices used in this experiment, before and after incubation for 20 minutes in human serum, as shown in Fig.5. Comparing to the results of concentration measuring of the C5c in serum by the technique sandwich ELISA for medical devices used in this experiment, with the study of the surface structure of the same samples with the SEM technique, we observe that materials with greater activation of C5c factor is the Dacron and PTFE. Those were also the materials with the higher surface roughness comparing to the other samples. The Nitinol stent graft is the sample with the lowest roughness and it is, according to the measurement of the concentration of the C5c with sandwich ELISA, the material with the lowest activation of C5c of all endoprosthesis used in experiment. In this way, by comparing the results of measurements with different techniques, we demonstrated the direct correlation of the structure of biomaterial surface to the degree of activation of C5c factor in human serum.



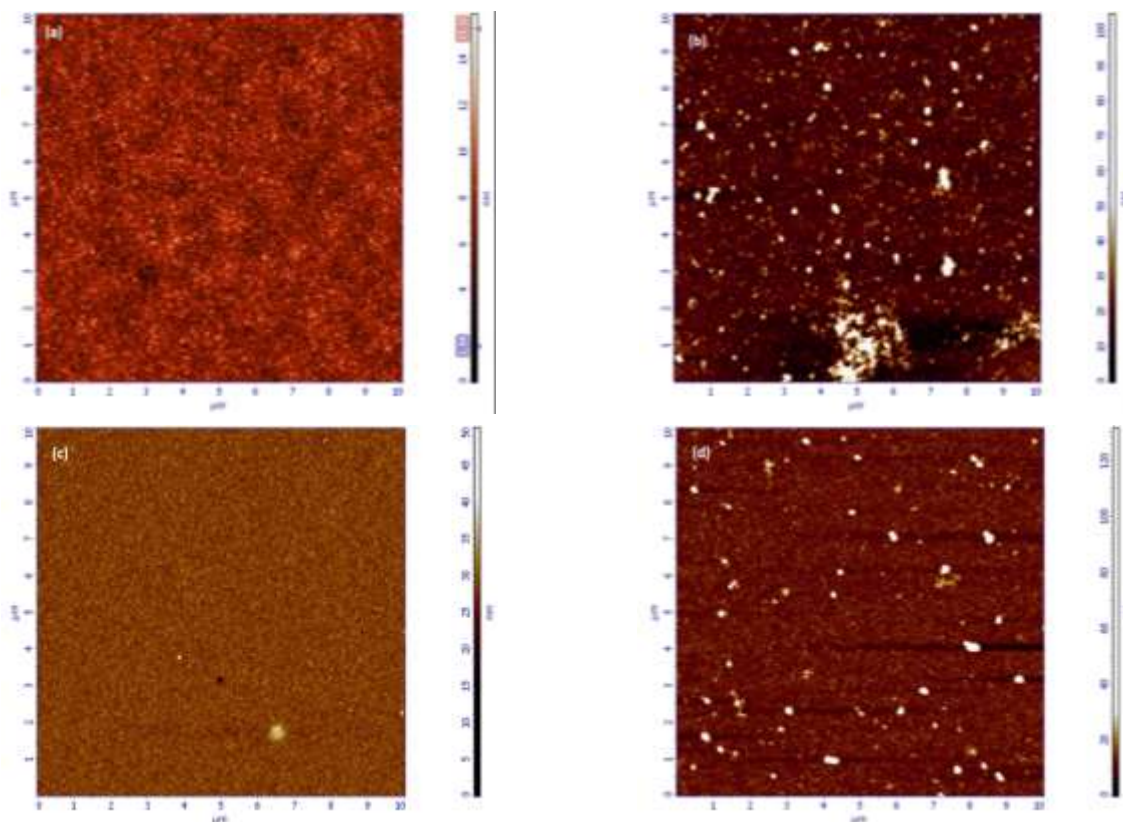
**Figure 5:-** Medical devices' surface topography by Scanning Electron Microscopy (SEM); (a) CoCr stent, (c) Nitinol stent graft and (e) PTFE before and (b) CoCr stent, (d) Nitinol stent graft and (f) PTFE after 20 min incubation in human serum.

### 3.3 Surface topography by Atomic Force Microscopy

The scanning of the samples was in the center of each sample (tapping mode for scan sizes of 10 and 5 mm) and the AFM images shown in 2D (Fig.6). Average roughness of each sample was calculated mainly from 10x10 micron images and the results are shown in Table I. The samples (Ti-Si, TiN-Si, a-C: H and Si) were examined before and after incubation of 20 minutes in human serum. The selection of the incubation time and the study of the samples topography were made after making the measurements of the ELISA kit activation C5c factor in serum. According to the ELISA kit measurements, after a period of 15-20 minutes of incubation, was observed stabilization in the kinetic of activation of C5c factor corresponding to complete coverage of the surface of the biomaterial and the gradual elimination of activated factor from serum. This was the reason why was chosen and the specific incubation time of 20 minutes for the topographic study of the samples. Knowing the topography of the sample it is possible to modify the above parameters of the surface [surface modification] that can provide the desired chemical, physical and biological properties of the biomaterial.

**Table I:-** Mean surface roughness of the thin films in this study before (in white font) and after incubation with serum for 20 min.

Groups	Root Mean Square (nm)	Peak to Valley (nm)
Ti	1.6	15.9
Ti_human serum	8.4	104.4
TiN	0.5	39.5
TiN_human serum	7.9	109.4
a-C:H	1.8	16.3
a-C:H_human serum	4.3	131.2



**Figure 6:-** AFM images of metal thin films (10x10mm); (a) Ti and (c) a-C:H before and (b) Ti and (d) a-C:H after the 20 min incubation in serum.

### 3.4 Contact Angle Measurements

For the measuring of contact angle (CA), the drop of deionized water was used. The samples were the metallic thin films: Ti, TiN, a-C:H and Si substrate. Each sample was measured three times at three different points and the contact angle is the average of three values obtained from each measurement. Using the contact angle measurements of the samples was calculated also another significant parameter, the free surface energy. The results are presented in the Table II. The Si substrate was used as a reference material and it was the material with the smallest contact angle and respectively the highest free surface energy of 54,8 (mN / m). The Ti metal thin film has low contact angle of  $58 \pm 0,7$  (deg) and correspondingly large free surface energy of 48,4 (mN / m). Generally, all samples exhibit hydrophilic behavior and there is no significant differences between the samples in the contact angle measurement. There is an inverse relationship between contact angle and free surface energy and the Table II showing this inverse relationship between the two sizes. Contact angle is inversely proportional to the free surface energy of the sample and with increasing of contact angle there is reducing of free surface energy and hence the ability of the sample to interact with polar solvents.

**Table II:-** Determination of the hydrophilicity / hydrophobicity of thin films with the technique of contact angle measurement

Thin Films	Contact Angle (deg)	Free surface energy $\gamma_{sv}$ (mN/m)
TiN	$64 \pm 0,2$	44,8
a-C:H	$70 \pm 0,2$	44,1
Si	$47 \pm 0,1$	54,8
Ti	$58 \pm 0,7$	48,4

### Discussion:-

In this study nanomaterials and already commercially available vascular devices were characterized in terms of their properties such as complement activation, surface morphology, topography and wetting behavior and their

correlation. Generally, uncontrolled complement activation can induce many inflammatory and life threatening conditions. The interaction between materials surfaces with the complement system remains complex and deserves special attention. The careful design and engineering behavior are therefore required to alleviate complement-related problems. The innovation in this study arises from two different aspects: i) material aspect: to assess the behavior of already known medical devices and metallic nanomaterials and to identify the responsible physicochemical properties and structural motifs that induces or not complement activation and ii) molecular aspect: to determine the activation measurement of a specific factor of the complement (C5c).

From a material's point of view, there are many geometrical and topological parameters at nanoscale that need to be considered when someone wishes to study the complement activation process. For example, the complement classical and lectin pathway convertase (C4b2a), has a cross-sectional diameter of approximately 30 nm [5]. This makes its assembly and deposition on to the surface of a nanomaterial with high surface area (e.g. nanofibres, nanoparticles) rather difficult [6]. Furthermore, a surface-bound C3b is expected to occupy an area of 40 nm<sup>2</sup> [7]. Thus, with very small complement activating nano-surfaces the bulk of activated C3 molecules will be released into the surrounding medium rather than being deposited on the nanomaterial's surface. Furthermore surface properties such as surface roughness, wettability and surface chemistry play an important role on protein adsorption, which is linked with protein adsorption, first biological event occurred on biomaterial surface, followed by cell adhesion and growth, when biomaterials are implanted in vivo or tested in vitro [8]. In this study, TiN thin films had lower surface roughness than Ti and a-C:H; with a-C:H having the highest surface roughness. After their incubation in serum, a-C:H demonstrated the lowest surface roughness indicating the least serum adsorption compared with the Ti and TiN. Ti thin films exhibited the highest surface roughness after the 20 min in contact with the serum. It is reported that nanoscale surface could affect protein adsorption and conformation [9], while surface roughness between 2 and 21 nm would not alter protein adsorption amount [10]. Although the surface roughness of the three materials was different, they were all below 6 nm and could be neglected.

From the contact angle measurements, it was shown that Ti was the most hydrophilic surface compared with TiN and a-C:H films. Generally, it is reported that a hydrophobic material surface is usually believed to tend to adsorb more protein than a hydrophilic material surface [8]. This comes into contradiction with the findings in this study, since the most hydrophilic surface demonstrated a higher adsorption rate (according to its surface roughness). There may be two reasons for this phenomenon of serum proteins adsorption. Firstly, serum contains many kinds of proteins, and different proteins may have different adsorption trends. It has been revealed that fibronectin showed greater adsorption on hydrophilic surfaces, whereas albumin predominantly adsorbed on hydrophobic surfaces [11]. Secondly, surface chemistry may play more important role in protein adsorption here. As reported by Clarke et al., surface roughness and hydrophobicity almost had no effect on albumin adsorption on NiTi alloy, while a clear correlation between surface nickel and oxygen concentration and amount of albumin adsorbed was observed [12]. Moreover, it has been reported that there is a favoured cell attachment on moderate hydrophilic surfaces, which permits the adsorption of serum proteins with labile and reversible bond [13]. The moderate degree of wettability of the substrates allows cells to deposit their own adhesion proteins, exchanging with the more rapidly adsorbed serum proteins [14].

On the other hand, from a molecular point of view, the complement system is involved in chronic inflammatory processes and is characterized as a very complex process. The significance of this study lies on the fact that the complement activation of the samples (nanomaterials and medical devices) was determined by the C5c in an attempt to link this factor with surfaces at nanoscale and improve their immunocompatibility. In other studies, the complement system is involved in chronic inflammatory processes and especially the components C3a and C5a exert strong chemotactic and proinflammatory effects [15-19]. Spiedl et.al. demonstrated increased complement activation as measured by higher levels of C3a and C5a before percutaneous coronary intervention is significantly associated with late lumen loss [20].

The choice of the incubation times in this study were based on the half-life of activation products in vivo. Selection of assays for use in clinical and experimental research [21,22] depends on various factors of which the half-life of activation products in vivo is essential. The highly active and important C5a fragment has a half-life of about 1 min [23,24], and might be difficult to detect in samples obtained in vivo. By contrast, various C3 activation products have half-lives of a few hours and are readily detectable [25,26].

**Conclusion:-**

The novelty of this study lied in selecting the activation measurement of the specific factor (C5c) of the complement as well as the materials to be studied. The effectiveness of the study is attributed on the fact that by measuring a single factor in serum, we acquired important information about the materials' properties. Therefore a comprehensive picture of the samples studied was observed. One of the goals of Nanomedicine is the potential applications of nanomaterials in medicine and effective control of biological reactions. The results indicate that the materials are excellent candidates for medical applications since they do not cause significant immune responses.

**References:-**

1. Mastellos D & Lambris JD. Complement: more than a "guard" against invading pathogens. *Trends Immunol* 2002;23(10):485-91.
2. Loos M. The complement system: activation and control. *Curr Top Microbiol Immunol* 1985;121:7-18
3. Mollnes TE, Song WC & Lambris JD. Complement in inflammatory tissue damage and disease. *Trends Immunol* 2002;23(2):61-64.
4. Rawal N, Pangburn MK. Structure/function of C5 convertases of complement. *Int Immunopharmacol* 2001;1(3):415-422.
5. Moghimi SM, Andersen AJ, Ahmadvand D, Wibroe PP, Andresen AL, Hunter AC. Material properties in complement activation. *Adv Drug Deliv Rev* 2011;63:1000-1007.
6. Andersen AJ, Hashemi AH, Andresen TL, Hunter AC, Moghimi SM. Complement: alive and kicking nanomedicines. *J Biomed Nanotechnol* 2009;5:364-372.
7. Janssen BJ, Christodoulidou A, McCarthy A, Lambris JD, Gros P. Structure of C3b reveals conformational changes that underlie complement activity. *Nature* 2006;444:213-216.
8. Yang D, Lü XY, Hong Y, Xi T, Zhang D. The molecular mechanism of mediation of adsorbed serum proteins to endothelial cells adhesion and growth on biomaterials. *Biomaterials* 2013;34:5747-5758.
9. Lord MS, Foss M, Besenbacher F. Influence of nanoscale surface topography on protein adsorption and cellular response. *Nano Today* 2010;5:66-78.
10. Cai K, Bossert J, Jandt KD. Does the nanometre scale topography of titanium influence protein adsorption and cell proliferation? *Colloids Surf B* 2006;49:136-44.
11. Wei JH, Igarashi T, Okumori N, Maetani T, Liu BL, Yoshinari M. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts. *Biomed Mater* 2009;4:045002.
12. Clarke B, Kingshott P, Hou X, Rochev Y, Gorelov A, Carroll W. Effect of nitinol wire surface properties on albumin adsorption. *Acta Biomater* 2007;3:103-11.
13. van Wachem PB, Bengeling T, Feijein J, Bantjes A, Detmers JP, van Aken WG. Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. *Biomaterials* 1985; 6:403-8.
14. Marques AP, Reis RL, Hunt JA. The biocompatibility of novel starch-based polymers and composites: in vitro studies. *Biomaterials* 2002;23:1471-1478.
15. Schulman ES, Post TJ, Henson PM, Giclas PC. Differential effects of the complement peptides, C5a and C5a des Arg on human basophil and lung mast cell histamine release. *J Clin Invest* 1988;81:918-23.
16. Nataf S, Davoust N, Ames RS, Barnum SR, Human. T cells express the C5a receptor and are chemoattracted to C5a. *J Immunol* 1999;162:4018-23.
17. Foreman KE, Vaporciyan AA, Bonish BK, et al. C5a-induced expression of P selectin in endothelial cells. *J Clin Invest* 1994;94:1147-55.
18. Okusawa S, Yancey KB, van der Meer JW, et al. C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro. Comparison with secretion of interleukin 1 beta and interleukin 1 alpha. *J Exp Med* 1988;168:443-8.
19. Bokisch VA, Muller-Eberhard HJ, Cochrane CG. Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator of human serum. *J Exp Med* 1969;129:1109-30.
20. Speidl WS, Katsaros KM, Kastl SP, Zorn G, Huber K, Maurer G et al. Coronary late lumen loss of drug eluting stents is associated with increased serum levels of the complement components C3a and C5a. *Atherosclerosis* 2010;208:285-289.
21. Harboe M, Thorgersen EB, Mollnes TE. Advances in assay of complement function and activation. *Adv Drug Deliv Rev* 2011;63:976-987.
22. Mollnes TE, Jokiranta TS, Truedsson L, Nilsson B, Rodriguez CS. Test, Complement analysis in the 21st century. *Mol Immunol* 2007;44:3838-3849.

23. Wagner JL, Hugli TE. Radioimmunoassay for anaphylatoxins: a sensitive method for determining complement activation products in biological fluids. *Anal. Biochem.* 1984;136:75–88.
24. Oppermann M, Gotze O. Plasma clearance of the human C5a anaphylatoxin by binding to leucocyte C5a receptors, *Immunology.* 1994;82: 516–521.
25. Teisner B, Brandslund I, Grunnet N, Hansen LK, Thellesen J, Svehag S.E, Acute complement activation during an anaphylactoid reaction to blood transfusion and the disappearance rate of C3c and C3d from the circulation. *J Clin Lab Immunol*1983;12:63–67.
26. Mollnes TE. Early- and late-phase activation of complement evaluated by plasma levels of C3d, g and the terminal complement complex. *Complement*1985;2:156–164.