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SiC nanoparticles as potential carriers for biologically active substances

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Abstract. Silicon carbide SiC thanks to its many advantageous properties has found numerous applications in diverse areas of technology. In this regard, its nanosized forms often with novel properties have been the subject of intense research in recent years. The aim of this study was to investigate the binding of biologically active substances onto SiC nanopowders as a new approach to biomolecule immobilization in terms of their prospective applications in medicine or for biochemical detection. The SiC nanoparticles were prepared by a two-stage aerosol-assisted synthesis from neat hexamethyldisiloxane. The binding of several proteins (bovine serum albumin, high molecular weight kininogen, immunoglobulin G) on SiC particle surfaces was demonstrated at the levels of 1-2 nanograms *per* mg of SiC. These values were found to significantly increase after suitable chemical modifications of nanoparticle surfaces (by carbodiimide or 3-aminopropyltrietoxysilane treatment). The study of SiC biocompatibility showed a lack of cytotoxicity against macrophages-like cells below the concentration of 1 mg nanoparticles *per* mL. In summary, we demonstrated the successful immobilization of the selected substances on the SiC nanoparticles. These results including the cytotoxicity study make nano-SiC highly attractive for potential applications in medicine, biotechnology or molecular detection.

1. Introduction

Recent advances in preparation of nanostructured composites with novel materials properties have stimulated many researchers towards investigation of their applications in various areas. Of special interest is the utilization of nanoparticles in bioanalysis, biotechnology, and medicine [1-6]. In this regard, the broad diversity of nanomaterials expands their potential applications. Metal nanoparticles are of considerable interest due to their optical, electronic, and catalytic properties while metal-like, magnetic or semiconductor nanoparticles offer other possibilities for bio-applications [6-8]. Among such materials, silicon carbide SiC presents a remarkable potential because of the suitable chemical inertness, hardness, high thermal stability, electroconductivity, *etc.* [9]. In particular, several nanosized materials forms have been evaluated for a variety of applications in medical sciences [10, 11].

The use of SiC in the micro-electro-mechanical system (MEMS) technology has been proposed [12, 13]. In contrast with other viable materials, *e.g.*, carbons, SiC due to its low chemical activity and high biocompatibility is an ideal candidate for surface coating of devices which can be in contact with tissues [14, 15]. The utilization of modified SiC thin films for serum protein adsorption or cell culturing suggests their potential usefulness for implant coatings [10, 11, 15, 16]. In this regard, barely reactive surfaces of this semiconductor often require modification for biomolecule binding [17-20]. The aim of this study was to investigate the binding of biologically active substances (proteins) onto SiC nanopowders obtained by a two-stage aerosol-assisted synthesis method. Subsequent chemical modifications of the particle surface were carried out to improve the magnitude of protein binding.

2. Materials and methods

2.1. *Materials*

SiC nanoparticles were obtained by the two-stage aerosol-assisted vapor phase synthesis from neat hexamethyldisiloxane (Sigma, USA) according to the published protocol [21] (av. crystallite size of

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β-SiC (Scherrer's equation), 3 nm; BET surface area (Gemini 2380, Micromeritics), 190 m^2/g). 3-aminopropyltriethoxysilane (APTES), biotin N-hydroxysuccinimide ester (NHS-biotin), bovine serum albumin (BSA), 1-(3-dimethyloaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC), N-hydroxysuccinimide (NHS), immunoglobulin G (IgG), phorbol 12-myristate 13-acetate (PMA), retinoic acid (RA), 3,3',5,5'-tetramethylbenzidine (TMB), and Tween-20 were purchased from Sigma Chemicals (USA). Horseradish peroxidase-conjugated streptavidin was purchased from MP Biomedicals (USA). High weight molecular kininogen (HK) was obtained from Enzyme Research Laboratories (USA).

2.2. *Adsorption of biotinylated protein on SiC nanoparticles.*
The proteins studied, BSA, HK, and IgG (0.04 mg/mL) were biotinylated with 50-fold molar excess of 30 mM NHS-biotin in 0.1 M $NaHCO_3$ buffer, pH 8.3, for 4 hours at 4 ºC. Labeled proteins were extensively dialyzed against phosphate buffer saline (PBS) and frozen at -20 ºC in 5 μL aliquots. Before protein adsorption tests, the SiC nanoparticles were treated in air at 700 ºC for 1.5 hours and then washed with 10 % HF for 0.5 hour. Subsequently, the powders were dried with vacuum pump. Protein solutions (100 μL) of concentrations in the range 0-5 μg/mL were incubated with 1 mg of SiC nanoparticles for 2 hours. After 3-fold washing with PBS supplemented with 0.1 % Tween 20, 200 μL of 1 % BSA solution were added to the SiC samples and incubated overnight at 4 ºC. Next, after 3-fold washing as above, the samples were transferred to new tubes and then treated with 100 μL of HRP-streptavidin solution (0.25 mg/mL) for 1 hour at room temperature. Finally, after washing, an enzymatic reaction was initiated with 0.01 % TMB in 0.05 M phosphate-citrate buffer, pH 5.0 supplemented with 0.006 % H_2O_2, and stopped with 100 μL of 1 M HCl. The aliquots were centrifuged and 200 μL of supernatants were transferred each to a microtitre plate for optical density measurements at 450 nm with Power Wave X Select reader (BioTek Instruments, USA).

2.3. *Chemical modifications of SiC nanoparticle surfaces*.
The first modification of SiC with NHS and EDAC was performed according to the modified two-step procedure of Jiang *et al.* [22]. After a 2-hour sonication of SiC nanoparticles (250 mg) in a H_2SO_4:HNO_3 solution (3:1 v/v) at 4 ºC, the sample was washed with distilled water to neutral pH and suspended in 1 mL of dioxane. The sample was subjected to modification according to the standard procedure [23]. NHS and EDAC *in substance* were added to get the respective 0.1 M concentrations and incubated for 1.5 hours with stirring at room temperature. Following an extensive washing with methanol the sample was dried. The second modification of the SiC surface with APTES was performed according to the standard procedure [24]. 250 mg of nanoparticles were boiled in 20 mL of 5 % HNO_3 for 45 minutes. The sample was washed with distilled water to get neutral pH and suspended in 20 mL of a 10 % APTES solution supplemented with 6 M HCl to reach pH equal to 3.5. The mixture was heated at 70 ºC for 3 hours. This was followed by an extensive washing with distilled water and, subsequently, the sample was dried. The surface-modified nanoparticles were analyzed by FT-IR with Nicolet 380 IR spectrophotometer (Thermo Electron Corporation, USA). Protein binding onto the SiC surface was measured according to the procedure specified above.

2.4. *Measurements of SiC nanoparticle cytotoxicity*.
The murine macrophage cell line RAW 264,7 and human macrophage-like cell line U937 used for the cytotoxicity analysis were from ATCC (USA). The cells were grown in RPMI medium, 2 mM glutamine, 25 mM HEPES (PAA Laboratories, Austria) supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin. The U937 cells ($5x10^5$ cells) were differentiated with 3 μM RA for 4 days until non-proliferating monocyte-like cells were obtained. Additionally, an overnight differentiation of these cells ($5x10^5$ cells) with 25 nM PMA was performed until adherent macrophage-like cells were obtained. The mice macrophages were grown until monolayers with approximately 70 % of confluence were obtained. The cells were incubated with SiC suspension with nanoparticle concentrations ranging from 0.01 to 1 mg/mL. After 1 or 2 days of incubation the

supernatants were separated by centrifuging. Nanoparticle cytotoxicity was estimated by the measurement of two metabolites released to culture medium: lactate dehydrogenase (LDH) and nitric oxide (NO). LDH levels were analyzed with CytoTox 96 kit (Promega, USA) and NO concentration were determined with a standard test [25].

3. Results

The applied SiC nanopowder was characteristic of the relatively high BET surface area of 190 m^2/g and small average crystallite size of 3 nm. The magnitude of the surface area is likely to be associated with the surface of the nanoparticles and intergranular system of pores. When comparing these values with the sizes of the applied proteins, *i.e.,* generally greater than 10-15 nm, it appears that adsorption of the bulky proteins is limited by the macro- and mesopores and involves aggregates of crystalline particles.

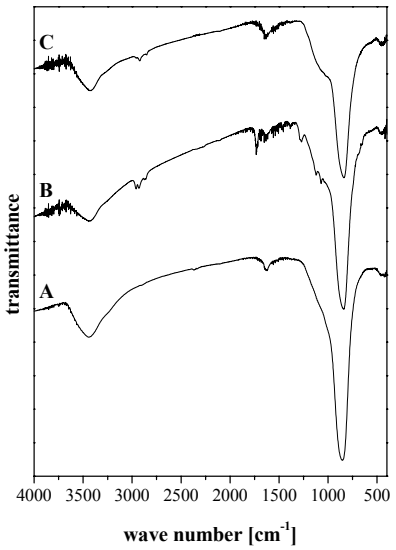

Figure 1. FT-IR spectra of SiC nanopowders: A – non-modified, B – after sonication in strong inorganic acids, C – after APTES modification.

Table 1. FT-IR band positions for SiC nanopowders: A – non-modified, B – after sonication in strong inorganic acids, C – after APTES modification.

A	B	C
425 (w)	449 (w)	453 (w)
856 (s)	845 (s)	837 (s)
	1070 (m)	1025 (sh)
	1123 (m)	
	1274 (w)	
	1388 (w)	
	1462 (w)	
1622 (w)	1629 (w)	1634 (w)
	1733 (w)	
	2864 (w)	2852 (w)
	2876 (w)	
	2929 (w)	2921 (w)
	2960 (w)	
3445 (m)	3446 (m)	3419 (m)

The IR spectra were recorded for the non-modified SiC powder and after its modification with APTES or after sonication with the concentrated acids (figure 1, table 1). The IR spectra for the non-modified nanoparticles showed the characteristic strong band for Si–C bonds at *ca.* 840 cm^{-1} and rather weak bands at *ca.* 3445 and 1622 cm^{-1} for the O-H bonds, the latter resulting from the specifics of preparation steps in the aqueous environment. The pair of the peaks associated with hydroxyl groups was also observed in all remaining samples [20]. After sonication in the presence of the concentrated inorganic acids several new bands appeared (table 1). The small intensity peaks at 1070 cm^{-1}/1123 cm^{-1} and 1388 cm^{-1}/1462 cm^{-1} are in the regions of the sulfate and nitrate group IR bands, respectively, supporting their presence in the material. Additionally, the small intensity peak at 1733 cm^{-1} is in the region observed for the C–O stretch in the carboxylic group suggesting some carbon species oxidation by the acid treatment (figure 1B). In the case of the APTES modification, the presence of the bands in the 2852-2921 cm^{-1} range and the well defined shoulder at *ca.* 1025 cm^{-1} suggest some adsorption of the modifying agent. Therefore, all powders surfaces (before and after modifications) contained various functional groups that, potentially, could interact with the proteins.

The amount of protein bound to the SiC nanoparticles was calculated from the linear standard curves obtained for the respective biotinylated protein with an identical procedure as for protein binding but carried out on the microtitre plate. The results were normalized to 1 mg of SiC nanopowder. The dependence of the amount of protein bound to 1 mg of SiC nanoparticles on the total

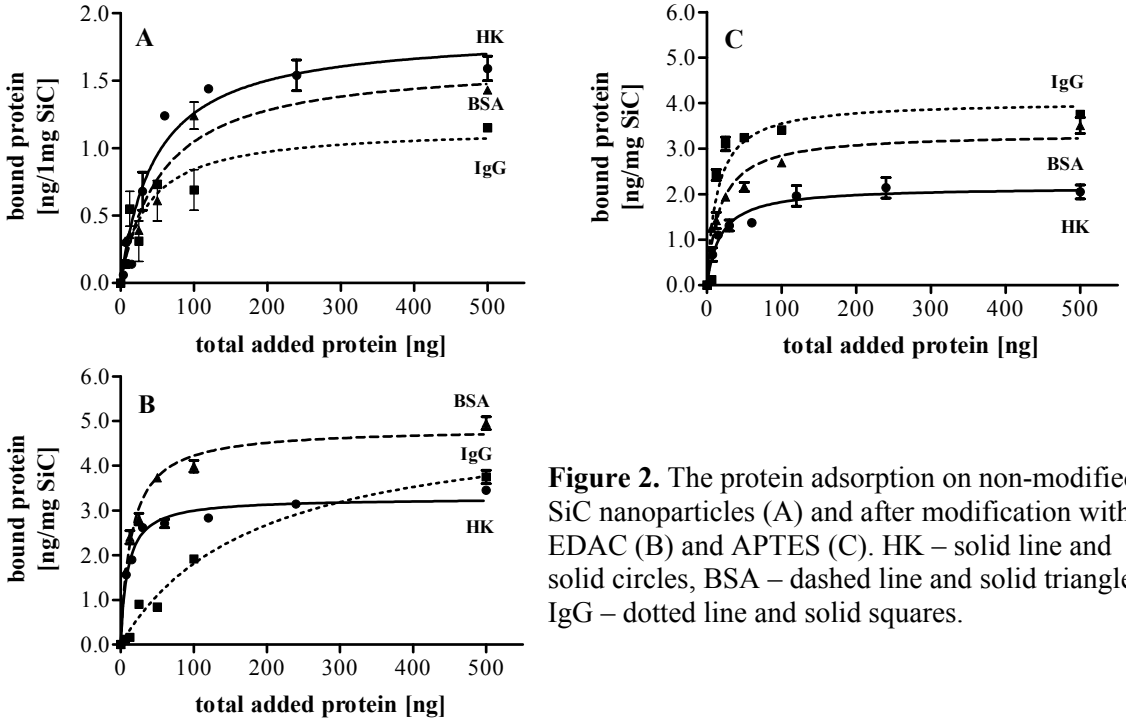

Figure 2. The protein adsorption on non-modified SiC nanoparticles (A) and after modification with EDAC (B) and APTES (C). HK – solid line and solid circles, BSA – dashed line and solid triangles, IgG – dotted line and solid squares.

amount of added protein is illustrated in figure 2A. All proteins showed a comparable adsorption on the SiC surface and saturation was apparently approached when approximately 250 ng of protein were added. The highest amounts of adsorbed protein varied from 1.15 ng for IgG to 1.59 ng for HK.

Modification of the SiC surface with EDAC and NHS significantly improved protein binding (figure 2B). The maximum amounts of the adsorbed protein varied from 3.45 ng for HK to 4.95 ng for BSA. The typical isotherm curve for IgG binding was different and no saturation within this concentration range was evident while saturation for the other proteins was observed at lower amounts of the total protein added (at 100 ng). The APTES modification enhanced the protein binding to the SiC nanopowders as compared to the non-modified SiC (figure 2C). The highest amounts of protein bound varied from 2.05 ng to 3.75 ng per mg of nanoparticles for HK and IgG, respectively. The binding saturation was reached at 100 ng of the total protein for all macromolecules.

The toxicity of SiC nanoparticles was examined for the concentration range 0.01-1.0 mg/mL. The biomarker of cytotoxicity, LDH, was not altered in monocyte-like cells U937 (figure 3A) while only for the highest SiC concentration after a 2-day incubation an increase of the LDL level was observed in macrophage-like cells (PMA-treated U937 and RAW 264,7 cell lines) (figures 3B, 3C). These results correlated with the NO level measurements (figures 3D, 3E, 3F). Increased amounts of NO were noted only for the incubation of the PMA-treated U937 cells and murine macrophages with a 1 mg/mL SiC suspension. This effect was more pronounced for a 2-day incubation.

4. Discussion

Adsorption of biomolecules on ceramic materials is a challenging field for researchers. In this study, the binding of three proteins on the surface of SiC nanoparticles was investigated. The as-prepared SiC nanopowders showed rather low adsorptive properties towards these proteins (up to 2 ng per mg SiC) compared with the previously reported studies for ceramic nanoparticles [26]. Also, the binding curves presented isotherm-like shapes with the saturation effect (figure 2A).

The surface modification of nanoparticles was performed to enhance the protein binding. The first type of modification, *i.e.*, with EDAC and NHS, was preceded by sonication in the presence of the

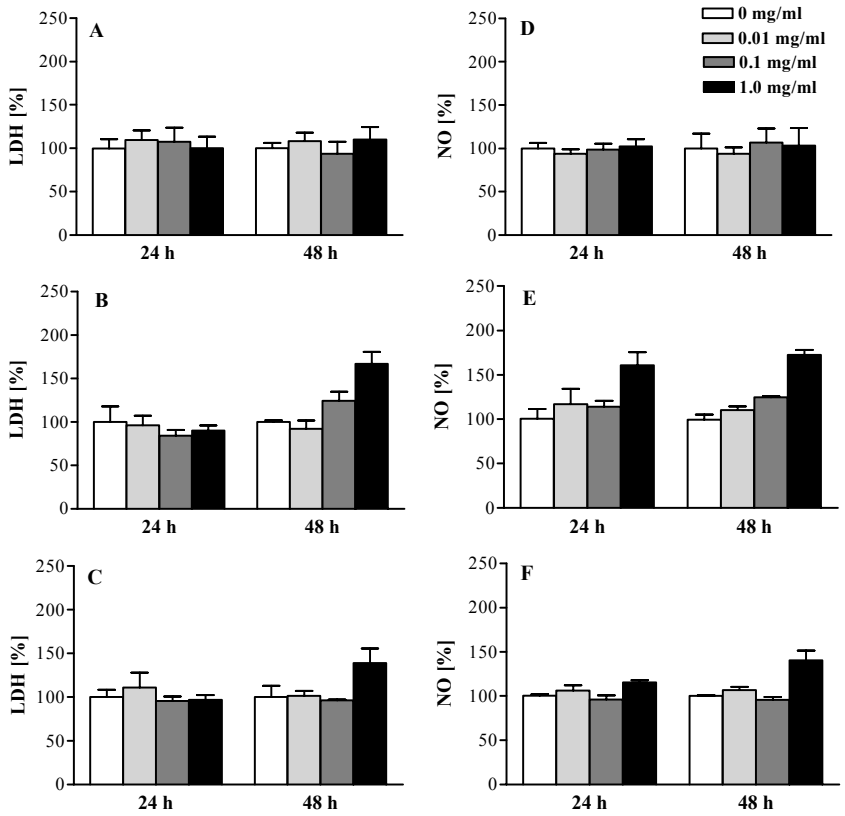

Figure 3. The cytotoxicity of SiC nanoparticles. Release of LDH and NO after incubation of SiC nanoparticles with: RA-treated cell line U937 (A, D); PMA-treated cell line U937 (B, E), and murine macrophage RAW 264,7 cell line (C, F). The graphs present the percentage of substance release in relation to untreated cells.

strong inorganic acids. The FT-IR spectra obtained after sonication in the acids displayed the band at 1733 cm^{-1} characteristic of carboxyl/carbonyl groups [19, 20] that supported some C-surface oxidation (figure 1B). The activated surface of SiC showed an increased protein-binding capacity (figure 2B). The acid proteins, BSA and HK, displayed a quick saturation effect with a slightly different extent of binding to SiC*, i.e.*, from 3-fold to 4-fold higher for BSA and HK, respectively, as compared with the non-modified SiC. On the other hand, the basic protein IgG did not saturate the EDAC-modified SiC nanoparticles. It is possible that due to the basic properties of IgG higher amounts of this protein were needed to achieve SiC surface saturation.

The APTES-modified nanoparticles showed the characteristic IR bands of aminoorganosilanes near 2921-2852 cm^{-1} for C-H and C-N bonds [19]. The shoulder near 1025 cm^{-1} is likely to be related to some APTES and/or residual Si-O bonds [19]. The silanization process seemed to be incomplete because the bands associated with the -OH groups did not disappear. The APTES modification introduced new amino groups to the surface and the nanopowder was more hydrophilic, hence, the acid proteins could bind more efficiently. That was the case of BSA when an increase of protein binding by 200 % was observed after modification (figure 2C). HK presented only a slight increase of binding while IgG was attached more abundantly to the APTES-modified SiC (*ca.* 4-fold). HK possesses a domain with high affinity towards negatively charged surfaces and, therefore, the amino group-saturated surface could not be ideal for its binding.

Some controversial information about toxicity of SiC nanoparticles has been reported [27, 28]. Those authors investigated toxic effects of SiC nanoparticles against several kinds of cells among others against monocytes or in macrophages. The observed difference were related to the amount of SiC used. In our study, we report no such effect on monocytes-like cells (figure 3) because neither

LDH nor NO increase are observed. On macrophage-like cells or on murine macrophages we confirm the earlier observations and only the highest SiC concentration after 48 hours of incubation shows a significant increase of the LDL and NO contents released to the culture medium.

In summary, we conclude that the applied β-SiC nanoparticles bind several model proteins with similar efficiency while suitable chemical modifications of the particle surface can significantly enhance the binding. These preliminary results, including the cytotoxicity study, qualify this inorganic nanopowder as attractive for potential applications in medicine, biotechnology or molecular detection.

5. References

[1] Jain K K 2005 *Clin. Chim. Acta* **358** 37-54
[2] Penn S G, He L and Natan M J 2003 *Curr. Opin. Chem. Biol.* **7** 609-15
[3] Fortina P, Kricka L J, Surrey S, Grodzinski P 2005 *Trends Biotechnol.* **23** 168-173
[4] Mirkin Ch A and Niemeyer Ch M 2007 Nanobiotechnology II. More concepts and applications (Weinheim: WILEY-VCH Verlag Gmbh)
[5] Jain K K 2008 The handbook of nanomedicine (New York: Humana Press)
[6] Mandal S, Phatare S, and Sastry M 2005 *Curr. Appl. Phys.* **5** 118-27
[7] Alivisatos A P 1996 *Science* **271** 933-37
[8] Henglein A 1989 *Chem. Rev.* **89** 1861-73
[9] Snead L L, Nozawa T, Katoh Y, Byun T-S, Kondo S, and Petti D A 2007 *J. Nucl. Mater.* **371** 329-77
[10] Auditore A, Satriana C, Coscia U, Ambrosone G, Parisi V and Marletta G 2002 *Biomol. Eng.* **19** 85-50
[11] Ilescu C, Chen B, Poenal D P, Lee Y Y 2008 S*ens. Actuators B* **129** 404-11
[12] Mehregany M and Zorman Ch A 1999 *Thin Solid Films* **355-356** 518-24
[13] Sarro P M 2000 *Sens. Actuators A* **82** 210-18
[14] Rosenbloom A J, Sipe D M, Shishkin Y, Ke Y, Devaty R P and Choyke W J 2004 *Biomed. Microdev.* **6** 261-67
[15] Gunn J and Cumberland D 1999 *Eur. Heart J.* **20** 1693-700
[16] Swain B P 2006 *Appl. Surf. Sci.* **253** 2310-14
[17] Zhang Y and Binner J 2002 *J. Am. Ceram. Soc.* **85** 529-34
[18] McMorrow B, Chartoff R, Lucas P, Richardson W and Anderson P 2006 *Compos. Interface* **13** 801-17
[19] Ji X, Hao H, Zhou B and Sun F 2007 *J. Wuhan Univ. Technol.- Mater.* **22** 754-56
[20] Wei G, Zhong S, Meng Y and Shu X 2007 *Plasma Sci. Technol.* **9** 57-61
[21] Czosnek C and Janik J F 2008 *J. Nanosci. Nanotechnol.* **8** 907-13
[22] Jiang K, Schadler L S, Siegel R W, Zhang X, Zhang H and Terrones M 2004 *J. Mater. Chem.* **14** 37-39
[23] Weetall H H and Filbert AM 1974 Porous glass for affinity chromatography applications *Meth. Enzym.* XXXIV ed W Jakoby and M Wilchek (New York: Colowick and Kaplan), ch. 4, p. 64
[24] Parikh I, March S and Cuatrecasas P 1974 Topics in the methodology of substitution reactions with agarose *Meth. Enzym.* XXXIV ed W Jakoby and M Wilchek (New York: Colowick and Kaplan), ch. 6, p. 86
[25] Guevara I, Iwanejko J, Dembińska-Kieć A, Pankiewicz J, Wanat A, Polus A, Gołąbek I, Bartuś S, Malczewska-Malec M and Szczudlik A 1998 *Clin. Chim. Acta* **274** 177-188
[26] Kim M I, Ham H O, Oh S, Park HG, Chang HN and Choi S 2006 *J. Mol. Catal. B: Enzym.* **39** 62-6
[27] Allen M, Butter R, Chandra L, Lettington A and Rushton N 1995 *Bio-Med. Mat. Eng.* **5** 151-59
[28] Nordsletten L, Kirsti A, Hogasen M, Konttinen Y T, Santavirta S, Aspennber P and Aesen A O 1996 *Biomaterials* **17** 1521-27

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