Amino Acids (2012) 43:823–831
DOI 10.1007/s00726-011-1138-1

ORIGINAL ARTICLE

Chemically modified diamond-like carbon (DLC) for protein enrichment and profiling by MALDI-MS

M. Najam-ul-Haq · M. Rainer · C. W. Huck · M. N. Ashiq · G. K. Bonn

Received: 3 May 2011 / Accepted: 25 October 2011 / Published online: 12 November 2011
© Springer-Verlag 2011

Abstract The development of new high throughput methods based on different materials with chemical modifications for protein profiling of complex mixtures leads towards biomarkers; used particularly for early diagnosis of a disease. In this work, diamond-like carbon (DLC) is developed and optimized for serum protein profiling by matrix-assisted laser/desorption ionization mass spectrometry (MALDI-MS). This study is carried out in connection with a material-based approach, termed as material-enhanced laser desorption ionization mass spectrometry. DLC is selected as carrier surface which provides large surface to volume ratio and offers high sensitivity. DLC has a dual role of working as MALDI target while acting as an interface for protein profiling by specifically binding peptides and proteins out of serum samples. Serum constituents are bound through immobilized metal ion affinity chromatography (IMAC) functionality, created through glycidyl methacrylate polymerization under ultraviolet light followed by further derivatization with iminodiacetic acid and copper ion loading. Scanning electron microscopy highlights the morphological characteristics of DLC surface. It could be demonstrated that IMAC functionalized DLC coatings represent a powerful material in trapping biomolecules for their further analysis by MALDI-MS resulting in improved sensitivity, specificity and capacity in comparison to other protein-profiling methods.

M. Najam-ul-Haq · M. Rainer · C. W. Huck (✉) · G. K. Bonn
Institute of Analytical Chemistry and Radiochemistry,
Leopold-Franzens University, Innrain 52a,
6020 Innsbruck, Austria
e-mail: christian.w.huck@uibk.ac.at

M. Najam-ul-Haq · M. N. Ashiq
Department of Chemistry, Bahauddin Zakariya University,
Multan 60800, Pakistan

Keywords Nanomaterials · Diamond-like carbon · IMAC · Protein profiling · MALDI-MS · Proteomics

Introduction

Proteomics is the research area in which various robust and reliable analytical techniques are employed for analyzing and tracking distinctive proteins which act as biomarkers in early detection and diagnosis of a disease (Etzioni et al. 2003; Seibert et al. 2005). Biomarkers are traced through different strategies like serum protein profiling, enzymatic digestions, peptide sequencing by tandem mass spectrometric methodologies (Abramovitz and Leyland 2006; Brusic et al. 2007). However, proteomics is still looking for biomarkers for clinical diagnostics and therapeutics (Dotzlaw et al. 2006; Morita et al. 2006). The analytical techniques widely employed are gel electrophoresis, chromatographic separations and coupling with mass spectrometric tools (Rehulka et al. 2005). The new strategies are to be built which can address the improvement in selectivity, sensitivity and specificity.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF–MS) (Zhao et al. 1991) is a sensitive detection tool for peptide and protein analysis. However, MALDI-MS without the pre-fractionation is limited in its ability. Therefore, through specially engineered materials and sample preparation strategies, it can be handier towards the pinpointing of disease markers. Studies have proved the use of MALDI in the revelation of disease biomarkers for all forms of cancer (Shahid et al. 2005; Pan et al. 2006). For instance, it is capitalized to distinguish prostate cancer from benign prostate hyperplasia and controls through pattern-matching algorithms. Therefore, the

tools like surface-enhanced laser desorption/ionization (SELDI) (Tang et al. 2003) or material-enhanced laser desorption/ionization (MELDI) (Feuerstein et al. 2006) are quite convincing for the detection of peptides and proteins from complex biological specimens. The developed materials in MELDI include carbon nanomaterials such as carbon nanotubes (Najam-ul-Haq et al. 2006a, b), graphitic nanofibres (Greiderer et al. 2009), C60 fullerenes (Vallant et al. 2007a, b), nanocrystalline diamond layers (Najam-ul-Haq et al. 2006a, b, 2008) and other chromatographic materials like cellulose (Feuerstein et al. 2005), silica (Trojer et al. 2005) and poly(glycidyl methacrylate/divinyl benzene) beads (Rainer et al. 2006, 2007). The MELDI approach comprises of an affinity probe for pre-concentration, extraction and screening of peptides and proteins from serum. The technique is a modification in MALDI-MS, where the normal target surface is replaced with affinity capturing sites. The sample preparation involves the loading of analytes after equilibration, incubation and washing, followed by the addition of photon absorbing matrix.

Nanotechnology in this regard brought technical innovations in life science research like in the area of biosensors, biomedical, drug delivery and biomarker research. The role of nanomaterials in the separation and bio-analysis is getting extensive. Their thermal, optical and electrical characteristics make them a sensitive solution in MALDI-MS analysis, due to their energy transferring capabilities (Guo et al. 2006). Particularly, carbon nanomaterials have received more and more attention in the past decade (Gruen 1999). Their nano sizes suffice the necessitated surface areas that are vital for characteristics like reactivity, capacity and sensitivity towards the laser energy. They are utilized for pre-concentration and provide affinity binding sites for proteins and oligonucleotides (Kong et al. 2005; Larsen et al. 2002).

Diamond-like carbon (DLC) is utilized in the form of surfaces and the surface properties are different than the bulk ones. DLC is a kind of amorphous carbon, which has few characteristics similar to diamond (sp^3 hybridization) (Robertson 2002). The major properties relevant to this study are inertness, smoothness and optical transparency. The inertness of DLC surfaces can withhold the most aggressive chemical environments. The benefit in DLC coatings is that it can be filmed on a variety of substrates where pure diamond is hard to coat due to elevated temperatures around 900°C during the CVD process. Regarding their efficiency, DLC surfaces show a combined effect rather than individual characteristics, because of the proper mixing of sp^2 bonded carbon atoms with sp^3 bonds. Due to these characteristics DLC is utilized in different areas of research (Hauert 2003). Particularly, biomolecules are selectively adsorbed on DLC surface using selective fabrication of hydrophobic and hydrophilic regions.

In this particular work, derivatized DLC surfaces deposited on various substrates like titanium and graphite are employed for their application in mass fingerprinting by MALDI-MS. The main focus is laid on immobilized metal ion affinity chromatography (IMAC) binding (Porath et al. 1975). The selection of chelating ligands (IDA) and loaded metal (Cu^{2+}) is based on their efficient specificity and adsorption capacity as both lead the group when compared with other available chelating agents and metals (Chaga 2001). The Cu^{2+}–IDA–DLC are treated with human serum and analyzed with MALDI-MS.

Experimental

Materials and reagents

All chemicals were used without further purification unless otherwise noted. Iminodiacetic acid (IDA, 98%), acetonitrile (ACN, for HPLC, ≥99.9%), isopropanol, glycidyl-methacrylate (GMA, for GC, ≥97.0%) and trifluoroacetic acid (for LC–MS, ≥99.9%) were purchased from Sigma-Aldrich (St. Louis, affinity binding sites MO, USA). Protein calibration standard-I and HCCA (α-cyano-4-hydroxy cinnamic acid) was bought from Bruker Daltonics Inc. (Bremen, Germany). All water used for preparing the standard solutions was purified by a NANO pure Infinity-unit (Barnstead, Boston, MA, USA). DLC coated on titanium was provided by Sony DADC (Anif, Austria).

Deposition of DLC surfaces

A titanium chip was washed with isopropanol and dried with nitrogen. The dried chip was immersed in isopropanol and ultrasonicated for 1 h. The DLC layer contained 35% sp^3 hybridisation which assures the mechanical stability and stiffness. The thickness of about 300 nm was prepared by pulsed laser desorption (PLD) technique (Bonelli et al. 2003). The layer conductivity was measured to be around 2 kΩ. A molybdenum interlayer with thickness of 30 nm was sputtered on top of the titanium layer using conventional sputtering equipment.

Scanning electron microscopy (SEM)

The diamond-like carbon surface was examined by SEM for its morphology after having cleaned with isopropanol. The surface was placed directly on stub without any additional conductor material. SEM's were performed in high-vacuum environment.

Derivatization of diamond-like carbon (DLC)

A DLC-coated surface on titanium substrate was washed with isopropanol and completely dried. The dried chip was placed in a specially designed inert chamber having a quartz window on top. Flow of nitrogen was maintained during the whole illumination time. 1 mL of glycidyl methacrylate (GMA) was mixed with 20 mg of azobisisobutyronitrile (AIBN) as a radical initiator for polymerization. The GMA–AIBN mixture was then added onto the surface of the chip in the form of a very thin layer and illuminated under ultraviolet (UV) light at 254 nm for 5 h through a quartz window. As soon as the thin layer of GMA polymer was formed on the DLC surface, it was taken out from the UV chamber. The viewable thin layer of GMA polymer was shiny and mechanically stable. Afterwards, the DLC chip was washed with 20 mL each of methanol and water and immersed in 50 mL of IDA solution; prepared by dissolving 10 g of IDA in 100 mL of 2 M sodium carbonate solution (pH 10), followed by the addition of 2 g sodium chloride. The chip was kept for 5 h at 75°C in IDA solution. Then the DLC chip was taken out, washed with 20 mL of acetonitrile and excess of deionized water until the washing solution was neutral. The chip was dipped into 100 mM copper sulfate solution for 2 h at room temperature. Finally the chip was washed with 20 mL of deionized water and dried under vacuum.

Derivatization of nanocrystalline diamond (NCD) surface

The NCD surface was derivatized according to the protocol already described (Najam-ul-Haq et al. 2006a, b). In brief, GMA was covalently attached to H-terminated NCD under UV light at 254 nm. The chip was then dipped in IDA solution, prepared by dissolving 10 g of IDA in 100 mL of 2 M sodium carbonate solution (pH 10), followed by the addition of 2 g NaCl. The chip was washed with deionized water and immersed in 100 mM copper sulfate solution for 2 h at room temperature.

Derivatization of diamond powder

Diamond powder was IMAC functionalized by first oxidizing it in a mixture of sulfuric acid and nitric acid (9:1) for 24 h. The resulting carboxylated diamond powder was reacted with thionyl chloride (10 mL) at 50°C for 24 h. The excess thionyl chloride was removed by rotary evaporator, and the powder was washed with dry THF. The amination of acid chlorides with IDA was carried out at 70°C for 90 h in the presence of triethyl amine. The excess of IDA was removed and washed with dichloromethane, ethanol and water. Finally, the material was loaded with copper(II) ions by adding 100 mM $CuSO_4$ solution for 2 h (Feuerstein et al. 2006).

Serum preparation

Raw serum was added into phosphate-buffered saline (PBS), pH 7.4 in the ratio of 1:5 and shaken for 10 min at 4°C. The diluted serum samples were kept in freezer (−20°C) to be engaged for sample preparation.

Sample preparation on IMAC-DLC and IMAC-NCD chips

The IMAC functionalized DLC surfaces were made more sensitive by introducing the anchor effects through a simplified approach. The 3-mm spots with regular succession on the modified DLC surface were gently covered. Teflon spray was coated in a very thin layer form. After the air-drying, the coverings were removed and DLC surfaces were ready for the sample preparation.

The binding tendency on bare DLC surface was checked by adding 40 μL of raw serum sample. After incubating for 1 h, washing with three times PBS buffer and once with deionized water was carried out. Before mass spectrometric analysis 1 μL of saturated HCCA in 50% acetonitrile and 0.1% trifluoroacetic acid (TFA) was added on the sample spot. For specific binding on Cu^{2+}-functionalized DLC and Cu^{2+}-functionalized NCD, the surfaces were first activated with 30 μL of 50 mM sodium acetate buffer and equilibrated with 30 μL of PBS. Afterwards 30 μL of raw serum sample was added on the chip and incubated on a platform shaker for 30 min. The non-specifically bound serum constituents were washed three times with 30 μL of PBS and once with 30 μL of deionized water. Finally, 1 μL of HCCA solution was applied to each spot for MALDI-MS analysis.

Sample preparation on IMAC-diamond powder

Three milligram of modified diamond powder was activated with 50 mM sodium acetate buffer for 5 min at room temperature and centrifuged. The residue was equilibrated with 200 μL of PBS buffer. 400 μL of serum sample was incubated for 2 h. The un-specifically bound proteins were washed with PBS buffer, followed by washing with deionized water. 1 μL of protein-loaded IMAC-diamond powder was spotted on the MALDI plate followed by the addition of 1 μL HCCA solution for MS analysis.

MALDI/TOF–MS analysis

The bound proteins were analyzed by MALDI/TOF–MS (Ultraflex-II, MALDI-TOF–TOF, Bruker Daltonics, Bremen,

Germany). The mass profiles were obtained in linear mode with 337 nm nitrogen laser having 3 ns laser pulses at the detector energy of 1,623 V. The laser pulse repetition rate was adjusted to 50 Hz. The mass spectra were generated by averaging 300 laser shots through the conventional analysis approach that includes the spot-to-spot analysis by searching the sweet spots manually through "stop and go" strategy. The validation of all the data, including baseline subtraction, external calibration using Protein Standard I (Bruker Daltonics, Bremen, Germany) and all further data processing was carried out by Flex analysis 2.0 post analysis software and data acquisition by Flex control 2.0.

Results and discussion

DLC for protein profiling

The functionalization of an inert material like DLC brings the specific adherence of biomolecules out of the serum samples. DLC is employed for this application in the form of thin coatings. The bindings occur primarily through the loaded Cu^{2+} ions and additionally van-der-Waals interactions also contribute towards protein-binding after prolonged periods of incubation. That is why in this study, well-optimized sample preparation protocols are employed. The fractionation is based on the differences in the affinity of target molecules towards the metal ions. This particular work involves copper (Cu^{2+}) as loaded metal on metal chelating IDA. Cu^{2+} belongs to the intermediate metal ions and has the coordinating potential towards nitrogen, sulfur and oxygen. Cu^{2+} particularly binds the amino acids of peptides and proteins at N-terminals (Andersson and Sulkowski 1992).

Scanning electron microscopy (SEM)

The IMAC functionalization coverage on DLC surface is a subject of overall surface area. The surface smoothness is preferred during the DLC deposition procedures to provide homogeneity to the chemical functionalization. Various forms of DLC layers with varied ratios of sp^2 and sp^3 content are checked; however, the DLC layer with 65% graphitic sp^2 suffices the conductivity requirements necessary in desorption/ionization mechanisms. There is a very thin uniform layer of GMA polymer created through UV polymerization. The uniformity depends a lot on the smoothness of DLC surface. The smooth homogenous morphology and deposition patterns are evident in the SEM picture (Fig. 1).

The surface under the desired magnification looks a closed surface, which offers required stability in UV-assisted chemical functionalization. As if the surface is not properly closed, it can trigger the phenomenon where liquid reagents penetrate through those cracks, lift the surface away and expose the underlying metal interlayer or the substrate material. Furthermore, it is observed that the improperly closed DLC layers when incorporated in the MALDI-MS system after sample preparation take longer times to establish the vacuum. This is attributed to the fact that the sample and utilized buffers penetrate beneath the DLC layer and hence hinder the evacuation times required to run the MALDI-MS analysis. The impact is also observed in the loss of sensitivity and spectral parameters lack in reproducibility.

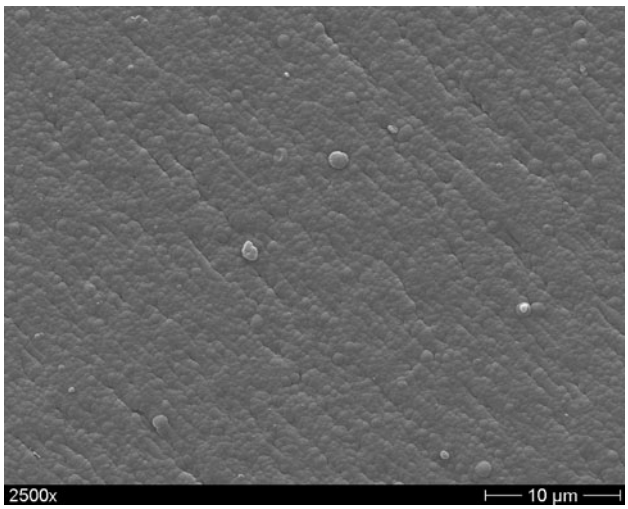

Fig. 1 SEM picture of smooth DLC surface, ensuring the closeness and homogeneity of coatings

DLC surfaces as MALDI targets

The MS measurements are improved by engineering the target surfaces with an appropriate conductivity. The conductivity is achieved by adjusting the sp^2 to sp^3 ratios in the DLC surfaces. Furthermore, molybdenum metal interlayer is present in between, which improves the thermal and electrical properties to an extent vital in desorption/ionization mechanism. The interlayer also brings optimal thickness to the target and thus the re-focus of the laser beam is not required. The DLC surfaces simultaneously act as MALDI target and also offer affinity sites for screening blood serum samples by specifically binding peptides and proteins. Furthermore, the sensitivity is enhanced by incorporating the anchor effects. Anchors are created by the inherent hydrophilic chemical functionalization, surrounded by a hydrophobic material like Teflon. The sensitivity limit achieved is 100 amol/µL for a peptide standard.

Reproducibility

The IMAC functionalized DLC surfaces are checked for repeatability/reproducibility, after sample preparation with standard serum samples on derivatized DLC surfaces from different batches. Mass spectra a and b of Fig. 2 show completely similar mass fingerprint patterns. However, very minute differences in the protein profiles are caused by the small in-homogeneity of matrix crystallization. Ion signals in the recorded spectra reveal high capacity, as the peptides and proteins bound to the IMAC-DLC surface are rich in number and provide better insight into the serum contents. The intensity of signals is high, which also shows the MALDI target properties of DLC surface as sensitive platform during the course of high throughput investigations of huge number of samples. In addition the signal qualities depict that the sample preparation protocols are effective, as there is no interference from salts present in the serum itself, which can hinder the desorption and ionization process in MALDI/TOF–MS. The batch-to-batch DLC derivatizations and the designed sample preparation protocols are highly repeatable as well as reproducible, which confirm the selectivity of surfaces in binding the targeted peptides and proteins.

Human serum albumin (HSA)

The IMAC-DLC surface is incubated with serum sample and analyzed around 66 kDa to check the binding feasibility of serum albumin. The serum albumin is attached to the derivatized IMAC-DLC. The albumin adsorption is pronounced around pH 7.0, which is also the incubating buffer (PBS, 7.4) pH range. The albumin-binding trend is verified because of its abundant nature in the serum. Many diagnostic markers are linked with serum albumin (Kang et al. 2005) and depletion of albumin might lead to a loss of potential candidates.

Comparison of bare and IMAC-DLC

The MALDI/TOF–MS investigations are carried out to find out if bare DLC (underivatized) shows any kind of physisorption phenomenon for peptides and proteins. Underivatized and derivatized DLC are loaded with serum samples and MALDI-MS spectra are recorded. As can be seen from Fig. 3a, the spectrum is rich in capacity due to the functionalized surface. The spectrum shown in Fig. 3b has limited peaks; however, there is an evidence of little physical adsorption for serum contents. The peaks are quite few in number, compared with the functionalized DLC

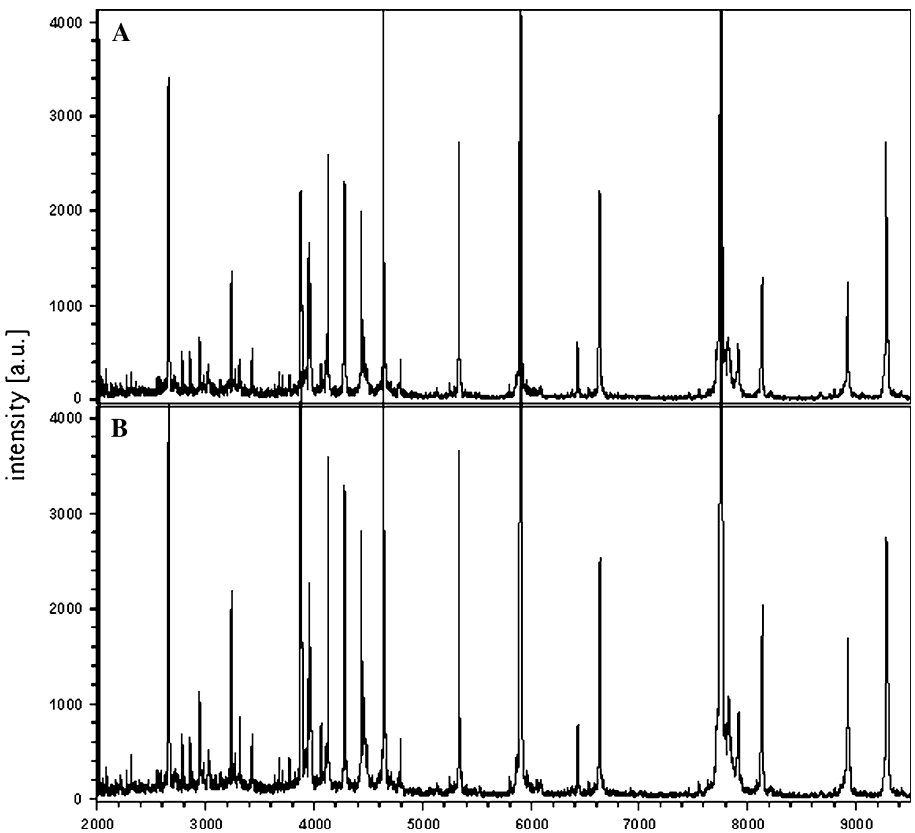

Fig. 2 MALDI/TOF–MS spectra of standard serum sample on IMAC-DLC support (35% sp^3) showing the reproducibility, from 2 to 10 kDa

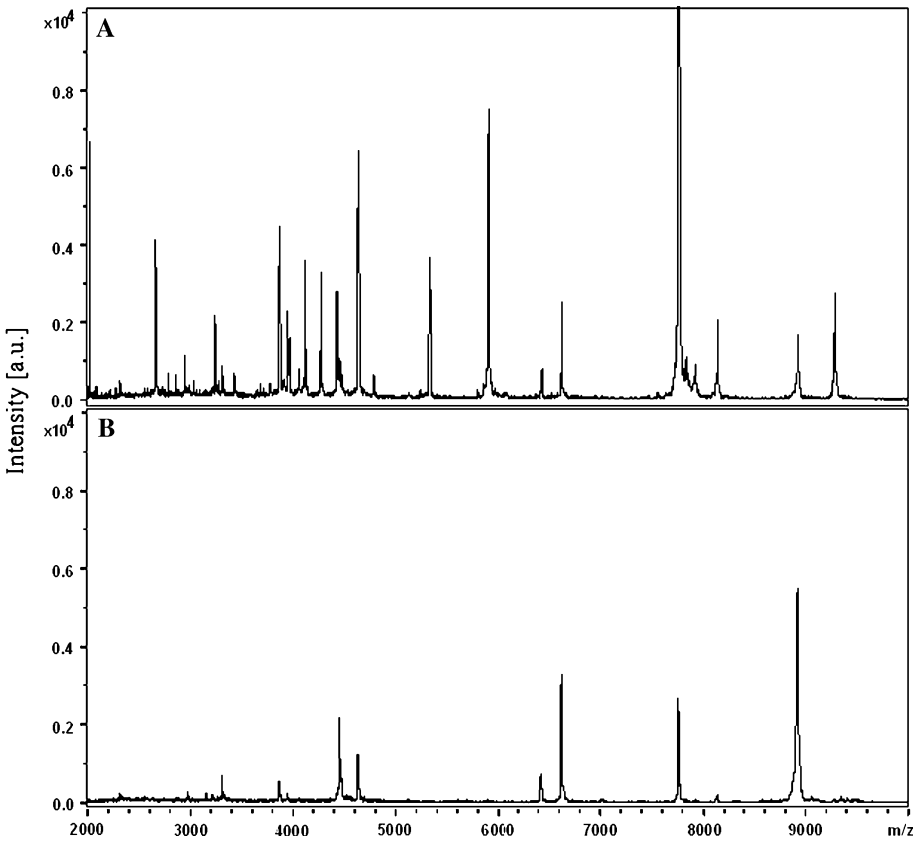

Fig. 3 Serum mass fingerprints on IMAC-DLC (**a**) and underivatized DLC (**b**) with MALDI/TOF–MS. The spectra are recorded in 10–20 kDa mass range with standard serum

surfaces. The reason behind the physisorption is based on the physical interactions like van-der-Waals, water ad-layer, polar and non-polar interactions.

The DLC-derivatized surface is also verified for any sort of background signals. The IMAC-DLC surface is gone through all the sample preparation protocols, including the equilibration and washing buffers except the incubation with serum sample. The routine matrix is added to the surface, so that any impurity content, if present, can be desorbed and ionized. There are no interfering background signals in MALDI-MS spectra from the functionalized surface. In addition, the surface and chemical functionalization are stable under the laser power, during desorption/ionization phenomenon.

Comparison of IMAC-DLC and IMAC-NCD

As the name implies, the DLC characteristics are similar to the pure diamond (NCD, nanocrystalline diamond, ~98% sp^3), because of the fair amount of presence of sp^3 hybridized carbons (35%). Therefore, the comparison of protein profiles of standard serum sample with same functionalization, i.e. IMAC-Cu^{2+} on NCD and DLC is carried out. The spectral profiles are different in their patterns and capacity, although the metal and chelating ligand are same. The higher capacity in case of DLC can be attributed to the presence of very thin polymer layer of GMA, derivatized with IDA and loaded with copper ions. This DLC surface situation is different from that of NCD, where GMA is covalently bound to the hydrogen-terminated NCD surface through UV and further functionalized with IDA and Cu^{2+} (Najam-ul-Haq et al. 2006a, b). This phenomenon also strengthens the MELDI viewpoint, where the material characteristics, in addition to the functionalities play a vital role in mass fingerprints. Furthermore, NCD surface is inert due to sp^3 carbons and thus resistant to chemical functionalization; however, the dangling bonds offer reactivity at higher energy configurations. Resultantly, the binding capacity of IMAC-DLC (a) is proved to be higher than that of IMAC-NCD (b), as shown in Fig. 4. Furthermore, not only the signals are more abundant, but also the overall *m/z* values are different among these two materials and hence different selectivity.

Comparison of IMAC-diamond powder with IMAC-DLC and IMAC-NCD surfaces

The comparison of three diamond-based nanomaterials, having the same functionalities, i.e. IMAC supports with same chelating ligand (IDA) and loaded metal (Cu^{2+}), is

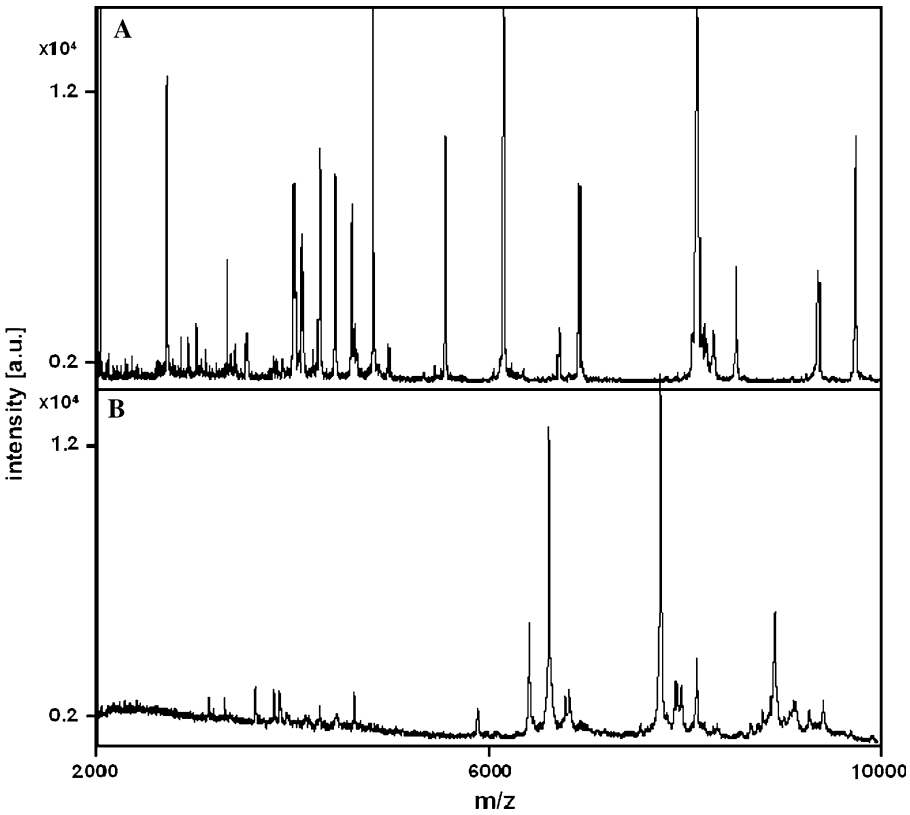

Fig. 4 Serum mass fingerprints on IMAC-DLC (**a**) and IMAC-NCD (**b**) with MALDI/TOF–MS. The spectra are recorded in 10–20 kDa mass range with standard serum

carried out to observe the finger printing patterns of standard serum sample aliquots in mass range from 2 to 10 kDa, according to the protocols described in "Experimental". The purpose is to observe the influence of nature of materials, which in this particular case has the part common, i.e. diamond (sp^3) nature. The investigated materials are NCD ($\sim$98% sp^3), DLC (35% sp^3) and diamond powder. All three materials are loaded with standard serum samples under the identical sample preparation protocols, except that diamond powder is a suspension system, whereas NCD and DLC are layer-based systems. Due to the same surface functionality used for all carrier materials (copper loaded IDA), the resulting mass fingerprints (Fig. 5) exhibited the same *m/z* values, except few marked differences.

The recorded protein profiles are brought to the same intensity levels for sake of clarity and comparison. Upon closer examination of the individual profiles in respective MELDI spectra, noteworthy differences are found which are marked. There are also minute differences in the overall intensity of these spectra, which can be due to the difference in surface conductivities, sample preparation and matrix crystallization. However, it is apparent from spectral profiles that a significant number of proteins are bound to these carrier materials, because of the adaptable range of hydrophilic functionalities and hydrophobic nature of materials.

Summarizing, the use of DLC-based MELDI is increasing the number of quality data derived from biological samples at the peptide and protein levels. It will have a major impact on basic biomedical research, as well as on the discovery of new drug targets and diagnostic markers. These carrier materials can simultaneously provide high-affinity sites to bind multitude of proteins in short period of time by employing the same buffer systems for sample preparation.

Conclusion

The DLC-based MELDI sample preparation is analyzing peptides and proteins specifically bound from serum samples with accurate and reproducible MS-traces. The SEM picture confirms the closeness and smoothness of DLC coatings. The DLC surfaces meet the sensitivity limits as they are known to possess energy-absorbing tendencies and hence simultaneously act as sensitive MALDI targets. The bonding sites are enhanced due to the higher surface areas. They show the potential to bind high-molecular-weight compounds like human serum albumin. The underivatized DLC surface shows hint of physisorption. The comparison with IMAC-NCD and IMAC-diamond powder reveals differences of some proteins present in one and absent in

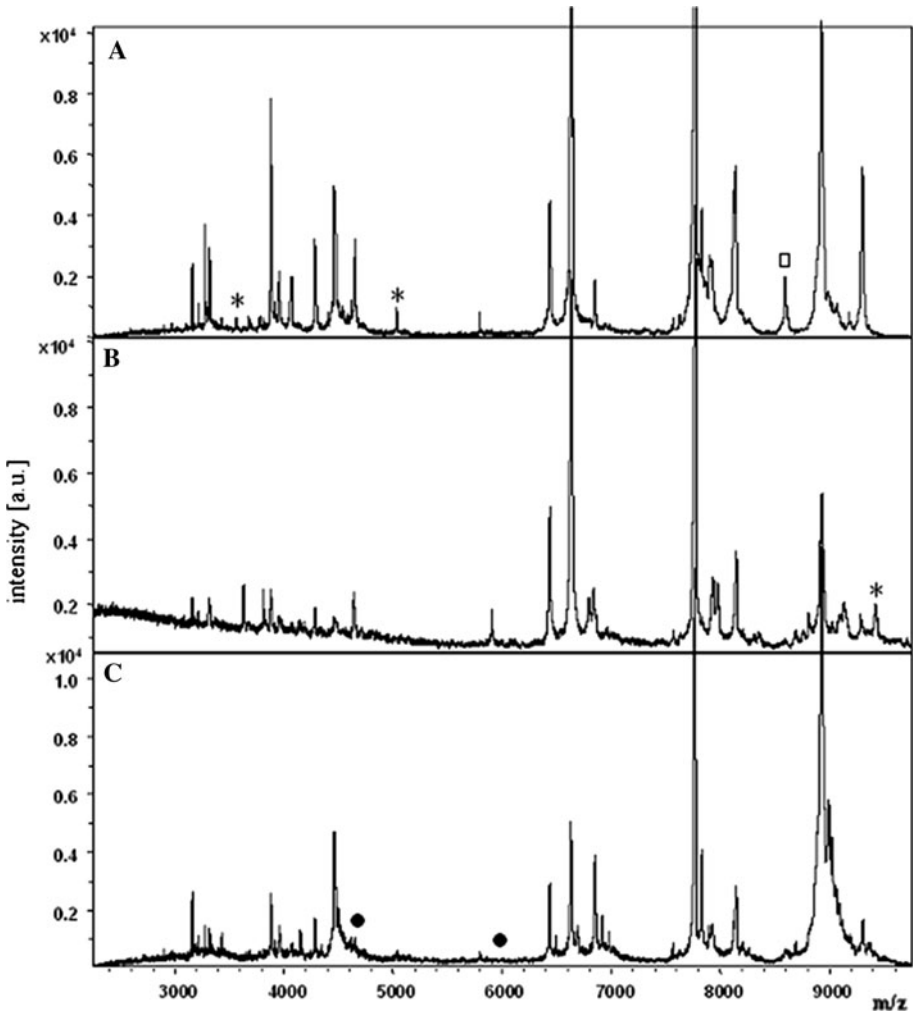

Fig. 5 MALDI/TOF–MS spectra of standard serum sample on IMAC-DLC (**a**), IMAC-NCD (**b**) and IMAC-diamond powder (**c**), from 2 to 10 kDa with 337 nm nitrogen laser. The differences among the profiles are marked

the other. Hence the performance and sensitivity levels are making IMAC-DLC surfaces much competitive to the state-of-the-art devices.

Conflict of interest Authors have no conflict of interest.

References

Abramovitz M, Leyland JB (2006) A systems approach to clinical oncology: focus on breast cancer. Proteome Sci 4:1–15. doi:10.1186/1477-5956-4-5

Andersson L, Sulkowski E (1992) Evaluation of the interaction of protein alpha-amino groups with M II by immobilized metal ion affinity chromatography. J Chromatogr A 604:13–17. doi:10.1016/0021-9673(92)85523-V

Bonelli M, Miotello A, Mosaner P, Casiraghi C, Ossi PM (2003) Pulsed laser deposition of diamond like carbon films on polycarbonate. J Appl Phys 93:859–865. doi:10.1063/1.1530725

Brusic V, Marina O, Wu CJ, Reinherz EL (2007) Proteome informatics for cancer research: from molecules to clinic. Proteomics 7:976–991. doi:10.1002/pmic.200600965

Chaga GS (2001) Twenty-five years of immobilized metal ion affinity chromatography: past, present and future. J Biochem Biophys Methods 49:313–334. doi:10.1016/S0165-022X(01)00206-8

Dotzlaw H, Eggert M, Neeck G, Schulz M (2006) Spots, blots, peaks and chips: proteomic approaches in autoimmune diseases. Curr Pharm Des 12:3699–3706. doi:10.2174/138161206778559704

Etzioni R, Urban N, Ramsey S, McIntosh M, Schwartz S, Reid B, Radich J, Anderson G, Hartwell L (2003) Early detection: the case for early detection. Nat Rev Cancer 3:243–252. doi:10.1038/nrc1041

Feuerstein I, Rainer M, Bernardo K, Stecher G, Huck CW, Kofler K, Pelzer A, Horninger W, Klocker H, Bartsch G, Bonn GK (2005) Derivatized cellulose combined with MALDI-TOF–MS: a new tool for serum protein profiling. J Proteome Res 4:2320–2326. doi:10.1021/pr050227z

Feuerstein I, Najam-ul-Haq M, Rainer M, Trojer L, Bakry R, Aprilita NH, Stecher G, Huck CW, Klocker H, Bartsch G, Guttman A, Bonn GK (2006) Material enhanced laser desorption/ionization (MELDI)—a new protein profiling tool utilizing specific carrier materials for TOF–MS analysis. J Am Soc Mass Spectrom 17:1203–1208. doi:10.1016/j.jasms.2006.04.032

Greiderer A, Rainer M, Najam-ul-Haq M, Vallant RM, Huck CW, Bonn GK (2009) Derivatized graphitic nanofibers (GNF) as a new support material for mass spectrometric analysis of peptides and proteins. Amino Acids 37:341–348. doi:10.1007/s00726-008-0159-x

Gruen DM (1999) Nanocrystalline diamond films. Ann Rev Mat Sci 29:211–259. doi:10.1007/s00726-008-0159-x

Guo Z, Ganawi AAA, Liu Q, He L (2006) Nanomaterials in mass spectrometry ionization and prospects for biological application.

Anal Bioanal Chem 384:584–592. doi:10.1007/s00216-005-0125-3

Hauert R (2003) A review of modified DLC coatings for biological applications. Diamond Relat Mater 12:583–589. doi:10.1016/S0925-9635(03)00081-5

Kang X, Xu Y, Wu X, Liang Y, Wang C, Guo J, Wang Y, Chen M, Wu D, Wang Y, Bi S, Qiu Y, Lu P, Cheng J, Xiao B, Hu L, Gao X, Liu J, Wang Y, Song Y, Zhang L, Suo F, Chen T, Huang Z, Zhao Y, Lu H, Pan C, Tang H (2005) Proteomic fingerprints for potential application to early diagnosis of severe acute respiratory syndrome. Clin Chem 51:56–64. doi:10.1373/clinchem.2004.032458

Kong XL, Huang LCL, Hsu CM, Chen WH, Han CC, Chang HC (2005) High-affinity capture of proteins by diamond nanoparticles for mass spectrometric analysis. Anal Chem 77:259–265. doi:10.1021/ac048971a

Larsen MR, Cordwell SJ, Roepstorff P (2002) Graphite powder as an alternative or supplement to reversed-phase material for desalting and concentration of peptide mixtures prior to matrix-assisted laser desorption/ionization-mass spectrometry. Proteomics 2:1277–1287. doi:10.1002/1615-9861(200209)2:9<1277:AID-PROT1277>3.0.CO;2-P

Morita A, Miyagi E, Yasumitsu H, Kawasaki H, Hirano H, Hirahara F (2006) Proteomic search for potential diagnostic markers and therapeutic targets for ovarian clear cell adenocarcinoma. Proteomics 6:5880–5890. doi:10.1002/pmic.200500708

Najam-ul-Haq M, Rainer M, Huck CW, Stecher G, Feuerstein I, Steinmüller D, Bonn GK (2006a) Chemically modified nano crystalline diamond Layer as material enhanced laser desorption ionisation (MELDI) surface in protein profiling. Curr Nanosci 2:1–7. doi:10.2174/157341306775473836

Najam-ul-Haq M, Rainer M, Schwarzenauer T, Huck CW, Bonn GK (2006b) Chemically modified carbon nanotubes as material enhanced laser desorption ionisation (MELDI) material in protein profiling. Anal Chim Acta 561:32–39. doi:10.1016/j.aca.2006.01.012

Najam-ul-Haq M, Rainer M, Heigl N, Szabo Z, Vallant RM, Huck CW, Engelhardt H, Bischoff KD, Bonn GK (2008) Nanostructured support materials, their characterization and serum protein profiling through MALDI/TOF–MS. Amino Acids 34:279–286. doi:10.1007/s00726-007-0492-5

Pan YZ, Xiao XY, Zhao D, Zhang L, Ji GY, Li Y, Yang BX, He DC, Zhao XJ (2006) Application of surface-enhanced laser desorption/ionization time-of-flight-based serum proteomic array technique for the early diagnosis of prostate cancer. Asian J Androl 8:45–51. doi:10.1111/j.1745-7262.2006.00103.x

Porath J, Carlsson J, Olsson I, Belfrage G (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258:598–599. doi:10.1038/258598a0

Rainer M, Najam-ul-Haq M, Huck CW, Feuerstein I, Bakry R, Huber LA, Gjerde D, Zou X, Qian H, Du X, Wei-Gang F, Ke Y, Bonn GK (2006) Ultra fast mass fingerprinting by high-affinity capture of peptides and proteins on derivatized poly(glycidyl methacrylate/divinylbenzene) for the analysis of serum and cell lysates. Rapid Commun Mass Spectrom 20:2954–2960. doi:10.1002/rcm.2673

Rainer M, Najam-ul-Haq M, Bakry R, Huck CW, Bonn GK (2007) Mass spectrometric identification of serum peptides and proteins employing derivatized poly(glycidyl-methacrylate/divinyl-benzene) particles for selective immobilization and enrichment. J Proteome Res 6:382–386. doi:10.1021/pr060426y

Rehulka P, Chmelik J, Allmaier G (2005) The influence of nonspecific cleavage sites on identification of low molecular mass proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with seamless post-source decay fragment ion analysis. Rapid Commun Mass Spectrom 19:79–82. doi:10.1002/rcm.1750

Robertson J (2002) Diamond-like amorphous carbon. Mater Sci Eng R37:129–281. doi:10.1016/S0927-796X(02)00005-0

Seibert V, Ebert MPA, Buschmann P (2005) Advances in clinical cancer proteomics: SELDI-ToF-mass spectrometry and biomarker discovery. Brief Funct Genom Proteom 4:16–26. doi:10.1093/bfgp/4.1.16

Shahid M, Selma U, Parkinson E, Schlenzka I, Dryden I, Lee L, Ball G, Colin C, Robert R, Schadendorf D (2005) Serum proteomic fingerprinting discriminates between clinical stages and predicts disease progression in melanoma patients. J Clin Oncol 23:5088–5093. doi:10.1200/JCO.2005.03.164

Tang N, Tornatore P, Weinberger SR (2003) Current developments in SELDI affinity technology. Mass Spectrom Rev 23:34–44. doi:10.1002/mas.10066

Trojer L, Stecher G, Feuerstein I, Bonn GK (2005) Cu(II)-loaded iminodiacetic acid/silica particles for protein profiling of human serum samples using surface-enhanced affinity capture: support porosity effects. Rapid Commun Mass Spectrom 19:3398–3404. doi:10.1002/rcm.2205

Vallant RM, Szabo Z, Bachmann S, Bakry R, Najam-ul-Haq M, Rainer M, Heigl N, Petter C, Huck CW, Bonn GK (2007a) High throughput solid phase extraction (SPE) of biomolecules employing C60 derivatized silica. Anal Chem 79:8144–8153. doi:10.1021/ac0712392

Vallant RM, Szabo Z, Trojer L, Najam-ul-Haq M, Rainer M, Huck CW, Bakry R, Bonn GK (2007b) A new analytical material-enhanced laser desorption ionisation (MELDI) based approach for the determination of low-mass serum constituents using fullerene derivatives for selective enrichment. J Proteome Res 6:44–53. doi:10.1021/pr060347m

Zhao S, Somayajula KV, Sharkey AG, Hercules DM, Hillenkamp F, Karas M, Ingendoh A (1991) Novel method for matrix-assisted laser mass spectrometry of proteins. Anal Chem 63:450–453. doi:10.1021/ac00005a012