Proteomic profiling of human bone from different anatomical sites – A pilot study

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Funding information
Deutsche Forschungsgemeinschaft,
Grant/Award Numbers: SCHI 871/17-1, NY 90/6-1, SCHI 871/15-1, GR 4553/5, A 2807/3-1, INST 39/1244-1 (P12), INST 39/766-3 (Z1)

Abstract

Purpose: The study aim is a comparative proteome-based analysis of different autologous bone entities (alveolar bone [AB], iliac cortical [IC] bone, and iliac spongiosa [IS]) used for alveolar onlay grafting.

Experimental Design: Site-matched bone samples of AB, IC, and IS were harvested during alveolar onlay grafting. Proteins were extracted using a detergent-based (sodium dodecyl sulfate) strategy and trypsinized. Proteome analysis was performed using liquid chromatography–tandem mass spectrometry (LC-MS/MS). MaxQuant was used for peptide-to-spectrum matching, peak detection, and quantitation. Linear models for microarray analysis (LIMMA) were used to detect differentially abundant peptides and proteins.

Results: A total of 1730 different proteins were identified across the 15 samples at a false discovery rate of 1%. Partial least-squares discriminant analysis approved segregation of AB, IC, and IS protein profiles. LIMMA statistics highlighted 66 proteins that were more abundant in AB then in IC (vs. 92 proteins were enriched in IC over AB).

Abbreviations: AB, alveolar bone; IC, iliac cortical bone; IS, iliac spongiosa; ECM, extracellular matrix; LC-MS/MS, liquid chromatography–tandem mass spectrometry; SRQR, Standards for Reporting Qualitative Research; SD, standard deviation; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; FDR, False discovery rate; EBInstitute of Bioinformatics Institute; LIMMA, Linear models of microarray analysis; PLS-DA, Partial least-squares discriminant analysis; GO, Gene Ontology; POSTN, mimecan; MPO, myeloperoxidase; ELANE, neutrophil elastase; AZU1, azurocidin 1; HMGB1, high-mobility group box protein 1; MSCs, Mesenchymal stem cells; PSM, peptide-spectrum-matching; VCAN, Versican; ASPN, Asporin; Caspases, Cysteine-dependent aspartic-specific proteases; ITIH1, inter-alpha-trypsin inhibitor heavy chain H1; BGN, Biglycan (BGN); AHS, alpha-2-HS-glycoprotein; ATP5A1, ATP synthase F1 subunit alpha; PRDX6, peroxiredoxin-6; DC, dendritic cells; VEGF, Vascular endothelial growth factor.

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Proteomics Clin. Appl. 2022;16:2100049. www.clinical.proteomics-journal.com
https://doi.org/10.1002/prca.202100049
INTRODUCTION

The loss of alveolar bone (AB) due to maxillofacial trauma, tumor resection, genetic disposition, periodontal, or dental disease can be reconstructed by bone grafting and subsequent implant placement [1]. When compared to bone substitutes, autologous bone grafts remain the gold standard as they exhibit osteoconductive, osteoinductive, and osteogenic properties [1, 2]. In oral- and maxillofacial surgery, autologous bone is harvested routinely from the mandible (AB) or the iliac crest (iliac cortical [IC] and iliac spongiosa [IS]) [3]. For small-volume bone reconstruction, circumscribing volumes smaller than 5 mm in horizontal and vertical dimension, autologous bone grafts and bone substitute materials have proven successful [4–9]. However, in large-size alveolar defects (>5 mm) autologous bone grafts from the iliac crest become imperative [8, 10, 11]. To date the reason for the difference of the clinical performance of autologous bone grafts remains unknown but site-specific biological properties of the bone are speculated [8, 12–14].

Bone is a hierarchically organized organ involved in, for example, mineral ion homeostasis, energy metabolism, and hematopoiesis [15, 16]. Bone cell function is regulated by mechanobiologic and endocrine mechanisms and it is known to adapt to changes in a site-specific manner [17]. Phenotypic and functional differences of primary human osteoblasts derived from iliac and mandibular bone have been described [12, 13, 18, 19]. Wein et al. was able to demonstrate that primary human iliac osteoblasts compared to primary osteoblasts derived from the mandibula exhibit different cell- and angiogenic proliferative properties as well as differences in gene expression [18–20]. The measurement of mRNA levels does not allow a prediction of the protein concentration within a cell. Proteins are the principal mediators connecting gene expression and metabolism. Proteomic analysis allows the investigation of the full set of proteins that are produced by cells or organisms providing information about the current state of a cell [21]. In oral medicine, proteome analysis of the saliva and gingival crevicular fluid has been performed to discover candidate biomarkers for disease onset and progression mainly in periodontitis and oral squamous cell carcinoma research [22]. In the last decades, proteomics has had a low impact in skeletal research due to a technically demanding protein extraction from the bone matrix [21, 23, 24]. A variety of tools have been utilized for protein analysis including gas chromatography mass spectrometry, nuclear magnetic resonance spectroscopy, direct flow injection/liquid chromatography mass spectrometry, inductively coupled plasma mass spectrometry, and high-performance liquid chromatography [22]. In bone research, high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS)-based approaches are currently predestined methods for the identification of site-specific differences in protein composition [13, 21, 24, 25]. Proteomic analysis of the AB and transiliac bone samples of healthy individuals were performed but not within one subject [24–26]. Proteomic analysis was mainly used for comparisons of osteoporosis, osteoarthritis in long bones [26]. To date the proteomic profile of different matching skeletal sites within living healthy individuals has not been examined.

The aim of this pilot study is the comparative proteome-based analysis of various bone entities (AB, IS, and IC) to gain further insights into the differences of the clinical performance of the bone grafts in AB reconstruction.

MATERIAL AND METHODS

The study was approved by the ethics committee of the University Medical Center Freiburg, Germany (Ethik-Kommission Albert-Ludwigs-Universität, Freiburg, Votum 602/15). Before enrollment, the patients received information regarding the purpose of the study and signed an informed consent. All patients were consecutively enrolled between 2017 and 2018 in one study center meeting the inclusion criteria: patients with alveolar ridge atrophy in the upper or/and lower jaw with no systemic disease (e.g., osteoporosis, cancer) and no regular medication affecting bone homeostasis (e.g., bisphosphonates, glucocorticoids, antiresorptive agents, cyclosporines, thyroid hormones, medroxypregesterone acetates, and anticoagulants like warfarin and heparin). All patients were nonsmokers. In all patients, it was tooth loss followed by bone grafting and dental implant placement. In all cases, the teeth were lost many years before grafting. This study was performed in accordance with the Helsinki Declaration of 1964, as revised in 2013. The study was conducted in accordance with the Standards for Reporting Qualitative Research (SRQR) guidelines. A power analysis was not performed.

Bone harvesting

Bone samples of the AB, IC bone, and IS of five healthy female patients with a mean age of 57.8 years (standard deviation [SD]: 7.8, range: 43–
65 years) were harvested during iliac onlay bone grafting as described earlier [11]. The iliac bone was retrieved either from the right or the left anterior superior iliac crest, the AB specimens were harvested either from the maxilla or the mandible, depending on the site of grafting during contouring of the recipient graft site. Site-matched biopsies describe: a bone sample of the alveolar ridge and the iliac crest of one patient were harvested during one surgery. All surgical interventions were performed under general anesthesia. The biopsies were frozen in liquid nitrogen and stored at −80°C before grinding the bone for further analysis.

2.2 | Protein extraction method

The proteomic analysis was possible due to presence of surgical bone remnants after contouring the bone grafting. The same amount of bone powder (40 mg) was used for every sample. The proteins were extracted in 4 M guanidin hydrochlorid (G3272, Sigma–Aldrich, Taufkirchen), 30 mM Tris–HCl (T3253, Sigma–Aldrich), 50 mM ethylenediaminetetraacetic acid (EDTA 11278.01, Serva Electrophoresis GmbH, Heidelberg), and 15% v/v glycerol (G5516, Sigma–Aldrich) [27, 28]; pH 7.4. 40 mg of grounded bone was added to 1.4 mL extraction buffer with protease inhibitors (Protease Inhibitor Cocktail, Roche, Mannheim) and inserted into a 3.5 kDa slide A Lyzer Mini dialyses device (Slide-A-Lyzer MINI Dialysis Devices, 3.5 K, 2 mL, ThermoFisher Scientific, Rockford, USA), followed by dialysis against 44.5 mL of the extraction buffer at 4°C for 24 h; these steps were repeated twice. On the fourth day, the protein lysat was harvested. Protein concentration was determined by the bicinchoninic acid assay as commercialized by Pierce. Samples (100 µg each) were further prepared by in-solution digestion using trypsin and reduction/alkylation using DTT and iodoacetamide; followed by desalting using STAGE-TIPS. In data processing, median-centering of peptide and protein intensities was employed to adjust for minor differences with regard to loading and ionization.

2.3 | LC-MS/MS and data analysis

LC-MS/MS was performed with an Orbitrap XL mass spectrometer (Thermo Scientific) in combination with an Agilent 1200 nanoflow or an Easy Nano-LC 1000 (Thermo Scientific). m/z range was 370–2,000. The resolution has been set at 60,000 [29]. Data analysis was performed with MaxQuant (version 1.6.0.16). The human proteome database was the EBI human reference proteome, downloaded on September 1, 2020 (www.ebi.ac.uk/reference_proteomes/). For quantitative proteome comparison, no variable modifications were included. Label-free quantitation was employed, using the match-between-runs functionality. We included proteins whose identification and quantitation were based on single peptides but for further analyses such as quantitative comparison, we requested that a protein was identified and quantified in samples from at least four different patients per type of bone, for example, yielding a minimal identification coverage of eight different patient samples when comparing two types of bone. To probe for hydroxyproline modified peptides, hydroxyproline was included as a variable modification. To probe for endogenous proteolytic processing, semitryptic specificity was employed. False discovery rate (FDR) was set to 0.01. The iBAQ value was used to determine protein intensities. Linear models of microarray analysis (LIMMA) were used for statistical evaluation as described [30]. Partial least-squares discriminant analysis (PLS-DA, a supervised dimensionality reduction approach) was applied comparing AB, IS, and IC.

3 | RESULTS

3.1 | Patient cohort and protein identification

Bone (AB, IC, IS) from five donors each, yielding a total of three groups with five biological replicates each were investigated (patient data, Table 1). A total of 1730 different proteins were identified across the 15 samples. For an initial overview of protein composition, the study focused on those proteins that were identified and quantified in at least 80% of replicates per group, yielding a set of 396 proteins. For these, hierarchical clustering clearly separated the AB from the iliac bone (Figure 1A). PLS-DA corroborated segregation of the AB samples from the iliac bone samples and distinguished IC from IS samples (Figure 1B).

3.2 | Focused comparison of alveolar bone and iliac cortical crest

Further, a detailed evaluation of the different proteome composition between AB and IC was performed. A total of 457 proteins were identified and quantified in at least 80% of replicates in each group, now excluding any limitation imposed by proteome coverage of the IS samples. LIMMA statistics highlighted 66 proteins that were signifi-
**TABLE 1** Patient data

| Patient no | Systemic disease                                    | Medication      | Dental findings       | Reason for tooth loss | Denture          |
|------------|-----------------------------------------------------|-----------------|-----------------------|-----------------------|------------------|
| 1          | Stroke (2001)                                       | Ticlopidin      | Fully edentulous      | Periodontitis          | Removable denture|
| 2          | Hypertension, hypercholesterolemia                  | Lisipronil      | Fully edentulous      | Periodontitis          | Removable denture|
| 3          | Hypothyroidism                                      | No medication   | Partially edentulous  | Caries                | Removable denture|
| 4          | No systematic disease                               | No medication   | Partially edentulous  | Caries                | Removable denture|
| 5          | Hypertension, psoriasis, breast cancer (2013)       | Lisipronil      | Partially edentulous  | Caries                | Removable denture|

Significantly enriched (p-adjusted < 0.05) in AB over IC crest with a minimum increase that corresponds to a doubling of protein amount in AB (log2(IC/AB) > 1). Likewise, LIMMA statistics highlighted 92 proteins that were significantly enriched (p-adjusted < 0.05) in IC crest over AB (Figure 2A), with a minimum increase that corresponds to a doubling of protein amount in IC crest (log2(IC/AB) > 1). The quantitative global proteome overview underlines the existence of different proteome biologies in alveolar and iliac (cortical crest and spongiosa) bone. Prominently enriched hallmark proteins of AB include matrisomal proteins such as periostin (POSTN) or mimecan (OGN) as well as extracellular matrix (ECM) components such as collagen type XIV (COL14A1) (Supporting Information Tables S1 and S2). Prominently enriched hallmark proteins of IC crest include proteins highlighting inflammation and immune biology processes, such as myeloperoxidase (MPO), neutrophil elastase (ELANE), or azurocidin 1 (AZU1). The impression of a matrisomal versus an immune-related proteome fingerprint is substantiated by Gene Ontology (GO) enrichment analysis. The most notably enriched GO terms include ECM constituents for AB and a plethora of immune biology related terms for IC crest (Figure 2B). LIMMA statistics could not demonstrate any significant differences between IC and IS.

### 3.3 Hydroxyproline-modified peptides

Hydroxylation of proline residues ranks among the most predominant post-translational modifications of collagen proteins. An additional peptide-spectrum-matching (PSM) approach was performed with hydroxyproline as a variable modification in order to assess any differences in proline hydroxylation between alveolar and iliac (cortical crest and spongiosa) bone. In this peptide-centric analysis, we focused on hydroxy-proline bearing peptides that were identified and quantified in at least 66% of all replicates in each group and representing an overall localization probability >0.95. This approach yielded 257 hydroxyproline peptide. As can be expected, 95% of the hydroxyproline peptides map to collagen proteins. Their protein-normalized intensities are comparable in all three types of bone (Figure 3A). PLS-DA marginally segregates the types of bone based on protein-normalized, hydroxy-proline peptide intensity (Figure 3B). Of note, the X-variate 1 of the hydroxyproline PLS-DA (13%) is much smaller than the X-variate 1 of the protein-level PLS-DA (32%). Nevertheless, Kruskall-Wallis analysis indicated that there is a significant (p < 0.01) difference between sample medians when comparing the aforementioned hydroxy proline bearing peptide intensities. We employed Dunn’s post hoc with Bonferroni corrected p-values to identify significant intersample differences (Supporting Information Table S3).

Only three samples (AB1, AB2, IS3) show significant differences to more than two other samples, including samples of the same bone type. Despite the presence of some intersample differences, we conclude that systematic alterations of hydroxy-proline peptide intensities fail to emerge between the different bone types and that our findings rather indicate a comparable extent and pattern of hydroxy-proline modification in alveolar and iliac (cortical crest and spongiosa) bone.

### 3.4 Semi-tryptic peptides as indicators of endogenous proteolysis

In the present study, semitryptic PSM has yielded 175 semitryptic peptides which were identified and quantified in at least 66% of all replicates in each group. This set of near-ubiquitous semispecific peptides can serve as an indicator of endogenous proteolysis. Their protein-normalized intensities are comparable in the two types of iliac bone but noticeably less abundant in AB (Figure 4A). PLS-DA clearly segregates the different types of bone based on protein-normalized intensities of semitryptic peptides (Figure 4B) with an X-variate 1 that is comparable to the protein-level PLS-DA (32%). Kruskall–Wallis analysis indicated that there is a significant (p << 0.01) difference between sample medians when comparing the aforementioned semispecific peptide intensities. We employed Dunn’s post hoc with Bonferroni corrected p-values to identify significant intersample differences (Supporting Information Table S4). Samples AB2–AB5 displayed a significant difference to almost all IC samples and to many IS samples. Sample AB1 stands out by rather differing from AB3 and AB5, hence highlighting interindividual heterogeneity. We conclude that these findings point to systematic alterations of the intensities of peptides stemming from proteolytic truncation. In summary, endogenous proteolysis on AB is decreased in comparison to iliac (cortical crest and spongiosa) bone, possibly stemming from different levels of inflammatory proteases such as ELANE (gene = ELANE). >170 cleavage sites were ubiquitously found in AB and iliac crest (IC) as semitryptic peptides and were located between Val or Ala (Figure 5).
FIGURE 1  (A) Hierarchical clustering of the 396 proteins, which were identified in at least 80% of replicates in each group. The color key shows the median normalized iBAQ value. The clustering clearly separates AB from iliac bone (crest IC and spongiosa IS). (B) PLS-DA of the 396 proteins, which were identified in at least 80% of replicates in each group. X-variate 1: 32% explained variation shows a notable difference between alveolar and iliac bone. It also distinguishes IC crest and IS samples. AB, alveolar bone; IC, iliac cortical; IS, iliac spongiosa; PLS-DA, partial least-squares discriminant analysis

4 | DISCUSSION

The reason for the difference in clinical performance of autologous bone grafts in AB reconstruction is unknown. The comparison of intraoral and iliac bone using a proteomic analysis in humans has not been performed to date.

Using LC-MS/MS, a total of up to 1730 different proteins were identified in 15 samples (donor = 5). To date protein extraction of human AB has only been performed in two previously published proteome studies with a varying amount of extracted proteins (318 and 2625 proteins) [24, 25]. Bell et al. conducted a multiprotease digestion strategy (either a trypsin or a LysArgiNase digestion) and additionally examined the solid phase, which may explain the high final protein amount [25]. In the present study, a modified protein extraction method according to Dong et al. and Sroga et al. using trypsin digestion was used [27, 28]. Proteomic analysis of human osteonecrotic femoral heads showed a comparable proteome coverage as the present study [23]. Transcriptional or proteome profiling of different bone entities is limited to murine samples or gene expression analysis and small sample sizes. The proteomic analysis of different bone sites within one patient has not been performed to date [24, 25, 31, 32]. A standard digestion protocol for human bone proteomics has also not been established yet [21, 25].

A set of 396 proteins were identified and quantified in at least 80% of replicates per group (AB, IS, and IC). PLS-DA confirms a site-specific difference in proteome composition between iliac (cortical and spongious) and AB. LIMMA statistics of the data corroborated the site-specific differences of the two bone entities, since 66 proteins were significantly enriched in AB over IC and 92 proteins were significantly enriched in IC over AB (p-adjusted < 0.05). In the present study, enriched hallmark proteins of IC include proteins highlighting immune–inflammatory response, such as high-mobility group box protein 1 (HMGB1), MPO, ELANE, or AZU1. HMGB1 behaves as an important modulator between innate and adaptive immunity by attracting inflammatory cells, recruiting stem cells, and promoting their proliferation as well as activates dendritic cells (DCs) and promotes their functional maturation [33]. Moreover, HMGB1 stimulates MSCs to form and secrete VEGF, which may promote angiogenesis, neovascularization, and consecutive new bone formation around the graft [34, 35]. The simultaneous presence of MPO, ELANE, and AZU1 may indicate the presence of neutrophils. Neutrophils are the most abundant white blood cells in the blood stream and part of the innate immunity, they are crucial during the early phase of healing regulating osteoblast and osteoclast function besides detecting tissue damage and eliminating microbes [36, 37]. GO enrichment analysis in the present study revealed further high amounts of proteins of antimicrobial humoral response, defense response to bacteria, and positive regulation of the innate immune response. In summary, the data presents an immune-related proteome fingerprint of the iliac crest, which may be one parameter for the better clinical performance in large size bone augmentations [8, 38]. The interdependency of the immune and skeletal system has been described in orthopedic research [16]. The immunological processes are not only important during the early healing phase but also throughout the entire healing phase. The healing capacity and bone homeostasis depends on the experience of the individuals’ immune system [39]. The existence of a high number of immune-related proteins in the iliac crest might in part result in a higher osteoinductivity of the transplant when used as an autologous graft.

A major task of the AB is to absorb repetitive high masticatory forces transmitted by the muscles and the periodontal ligament of the teeth [40]. The evaluation of the proteins predominantly expressed in the AB compared to the bone from the iliac crest demonstrated a
FIGURE 2  (A) Volcano plot visualizing the quantitative proteome alterations when comparing AB to IC crest. Adjusted \( p \)-values of LIMMA statistics are plotted against mean protein fold changes. Our criteria for designating a protein as being significantly affected are visualized as dashed lines. The horizontal dashed line corresponds to \( p \)-adjusted = 0.05; the vertical dashed line corresponds to a 50% decrease or increase, respectively. (B) Bar-chart depicting GO enrichment results. The most notable GO terms include extracellular matrix constituents for AB and a plethora of immune biology related terms for IC crest. The dash line was set to 2. AB, alveolar bone; GO, Gene Ontology; IC, iliac cortical; LIMMA, linear models for microarray analysis.
FIGURE 3  (A) Illustration of hydroxyproline-containing peptides in alveolar and iliac crest bone. Boxplot (shown is 25th–75th percentile; the mean is marked as black horizontal line) of log2-transformed, protein-normalized intensities of hydroxyproline bearing peptides, occurring in at least 66% of replicate of each group. The plots of the samples are nearly equally, showing a comparable pattern of hydroxy-proline modification in alveolar (AB) and iliac (cortical crest and spongiosa) bone (IC and IS). (B) PLS-DA of log2-transformed, protein-normalized intensities of hydroxyproline bearing peptides, occurring in at least 66% of replicate of each group. The explained variation is low. This confirms the comparable pattern of hydroxy-proline modification in the different bone entities. AB, alveolar bone; IC, iliac cortical; IS, iliac spongiosa; PLS-DA, partial least-squares discriminant analysis.

FIGURE 4  (A) Illustration of semitryptic peptides in alveolar and iliac crest bone. Boxplot (shown is 25th–75th percentile; the mean is marked as black horizontal line) of log2-transformed, protein-normalized intensities of semitryptic peptides, occurring in at least 66% of replicate of each group. Only unique (proteotypic peptides) were considered, native N- and C-termini were excluded. The visualization shows the intensity of semitryptic peptides is comparable in iliac bone but less abundant in alveolar bone. AB, IC, and IS. (B) PLS-DA of log2-transformed, protein-normalized intensities of semitryptic peptides, occurring in at least 66% of replicate of each group. Only unique (proteotypic peptides) were considered, native N- and C-termini were excluded. The wide of 32% explained variation clearly segregates the different types of bone based on protein-normalized intensities of semitryptic peptides. AB, alveolar bone; IC, iliac cortical; IS, iliac spongiosa; PLS-DA, partial least-squares discriminant analysis.
higher expression of ECM structural constituents including collagen Typ VI alpha 1–3 (COL6A1, COL6A2, COL6A3), collagen type XII and type XIV alpha 1 (COL12A1, COL14A1), and mimecan (OGN), proteins involved in the endopeptidase inhibitor activity as well as cytoskeletal protein binding. The results are in accordance with the results of Salmon et al., which demonstrated a similar protein composition with high amounts of ECM proteins like Versican (VCAN), Asporin (ASPN), COL6A3, and Serpin H1 [24]. Likewise, Bell et al., who identified via LC-MS/MS ECM macromolecules like the hyaluronic acid-binding protein inter-alpha-trypsin inhibitor heavy chain H1 (ITIH1) and proteins which are involved in bone mineralization and formation like Biglycan (BGN) and alpha-2-HS-glycoprotein (AHSG) in the AB [25, 41]. Upon manual inspection, we also noticed that the AB-proteome shows proteins of the energy metabolism such as ATP synthase F1 subunit alpha (ATP5A1) and peroxiredoxin-6 (PRDX6). Findings of the PLS-DA corroborate segregation of the alveolar from the iliac bone samples indicating different biological properties. LIMMA statistics underlined the similarity between IC and IS (Supporting Information Table S2). The differences of the protein-composition between IC versus AB might be associated with the varying functional musculoskeletal demands [17]. The exhibited ECM-related proteome may serve the high mechanical forces experienced in AB. This difference in protein composition between iliac and AB suggests differential osteogenetic and osteoinductive properties when used as autologous bone transplants. It has been described that cells from different skeletal sites have distinct metabolic demands relating to their particular function and they are also affected by the environmental condition [19, 20, 42].

The hydroxyproline peptide intensities confirm the osseous origin of the investigated samples and are in accordance with the findings of Bell et al. [25]. The PSM for hydroxyproline was not performed by Salmon et al. [24]. The semitryptic peptides identified within this study are an indicator for endogenous proteolysis by secreted and cytosolic proteases, the semistable cleavage products are amenable to mass spectrometric analysis. Powerful methods exist for their specific enrichment [43, 44]. However, a first-line, low-coverage analysis is achievable by performing a semispecific (here: semitryptic) PSM, for example, as illustrated in a study of cardiac reverse remodeling [43]. In the present study, the protein-normalized intensities are comparable in IS and IC but are decreased in AB. The proteome comparison of AB and IC crest has indicated the enriched presence of inflammatory proteases such as neutrophil elastase or cathepsin G in IC. The elevated levels of endogenous proteolysis hint to the presence of proteolytic enzymes. An apoptotic proteolysis by caspases could be excluded in the present study since >170 cleavage sites, ubiquitously found in AB and IC as semitryptic peptides, are located between Val or Ala (Figure 5). Cysteine-dependent aspartic-specific proteases (Caspases) are known to orchestrate programmed cell death (apoptosis) and mediators of inflammation and cleave their protein substrates almost exclusively after D residue [43, 44].

A limitation of this pilot study is the small sample number and a lack of the power analysis due to the nature of a pilot study. Since only five females with a mean age of 58 years were included, the findings cannot be generalized to males and to other age groups. Two patients used antihypertensive medication which is being discussed controversial regarding its influence on bone metabolism, that is, bone mineral density [45–47]. Interindividual and site-specific differences found in protein composition and regulation should be verified in larger cohorts, characterizing the oral and general health of these patients with detail. Future studies could include information concerning medical reasons for tooth loss and ridge atrophy and provide clinical outcome of the
implant treatment. The observed interindividual differences may be influenced by the age of the individual as proposed by two recent studies in the field of forensics [48, 49]. However, in multiple studies, concerning the regeneration of defects or grafts or of in the AB, it was shown that a high rate of interindividual difference in new bone formation exists regardless of the age, this might be attributed to the fact that a plethora of factors interfere with bone physiology [42, 50–52]. Differences between alveolar and iliac bone may be influenced by the different embryonic origin of the bone. However, animal studies demonstrated that the survival and resorption rate of bone grafts is primarily determined by its graft composition rather than its embryonic origin [53]. This has been confirmed with current human data showing a long-term stability of the iliac bone graft when composed mainly of cortical bone [54]. Future proteomic-based studies should analyze a larger cohort and additional bone entities to decipher their biological potential for bone grafting in oral and maxillofacial surgery. The findings could help to improve therapeutic agents beyond single protein use by understanding the principal nature of AB biology.

5 | CONCLUSION

This pilot study demonstrated an ECM protein-related proteome fingerprint in AB and an immune-related proteome fingerprint in iliac bone based on shot-gun proteomics. Interindividual differences in protein composition were found, allowing first insights into the site- and patient-specific differences of bone transplants, seen in the clinical practice.

ACKNOWLEDGMENTS

The authors would like to thank Ute Hübner (I) for assistance during the study. Oliver Schilling acknowledges funding by DFG (SCHI 871/17-1, NY 90/6-1, SCHI 871/15-1, GR 4553/5-1, PA 2807/3-1, INST 39/1244-1 (P12), INST 39/766–3 (Z1)).

Open access funding enabled and organized by Projekt DEAL.

PATIENT CONSENT STATEMENT

This study was performed in accordance with the Helsinki Declaration of 1964, as revised in 2013. Before enrollment, the patients received information regarding the purpose of the study and signed an informed consent.

RESEARCH REPORTING GUIDELINES

The study was conducted in accordance with the SRQR (Standards for Reporting Qualitative Research) guidelines.

AUTHOR CONTRIBUTIONS

Conceptualization and idea, surgeries, validation, administration, and writing: Tobias Fretwurst and Katja Nelson. Proteomic analysis, statistics, review, and editing: Isabel Tritschler. Administration, review, and editing: René Rothweiler and Susanne Nahles. Proteomic analysis, statistics, review, and editing: Brigitte Altmann and Oliver Schilling.

CONFLICT OF INTEREST

The authors declared no conflicts of interest concerning the research, authorship, and/or publication of this article. This manuscript has not been published and is not under consideration for publication elsewhere. All authors gave their final approval and agree to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT

"LC-MS/MS data and analysis result files are available at the European Genome-phenome Archive* for appropriate research use (https://ega-archive.org; EGAS00001005809). As patient-centric proteomic data is increasingly regarded as sensitive, personal data, EGA requires adherence to a data access agreement. The data access agreement for this dataset corresponds to the "Harmonised Data Access Agreement (hDAA) for Controlled Access Data" as brought forward by the "European standardization framework for data integration and data-driven in silico models for personalized medicine – EUSTANDS4PM."

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Fretwurst, T., Tritschler, I., Rothweiler, R., Nahles, S., Altmann, B., Schilling, O., & Nelson, K. (2022). Proteomic profiling of human bone from different anatomical sites – A pilot study. Prot. Clin. Appl., 16, e2100049. https://doi.org/10.1002/prca.202100049