Patient-specific fracture risk assessment of vertebrae: A multiscale approach coupling X-ray physics and continuum micromechanics

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SUMMARY

While in clinical settings, bone mineral density (BMD) measured by Computed Tomography, remains the key indicator for bone fracture risk, there is an ongoing quest for more engineering mechanics-based approaches for safety analyses of the skeleton. This calls for determination of suitable material properties from respective CT-data, where the traditional approach consists of regression analyses between attenuation-related grey values and mechanical properties.

We here present a physics-oriented approach, considering that elasticity and strength of bone tissue originate from the material microstructure and the mechanical properties of its elementary components. Firstly, we re-construct the linear relation between the clinically accessible grey values making up a Computed Tomograph, and the X-ray attenuation coefficients quantifying the intensity losses from which the image is actually reconstructed. Therefore, we combine X-ray attenuation averaging at different length scales and over different tissues, with recently identified "universal" composition characteristics of the latter.

This gives access to both the normally non-disclosed X-ray energy employed in the CT-device, and to in vivo, patient- and location-specific bone composition variables, such as voxel-specific mass density, as well as collagen and mineral contents. The latter feed an experimentally validated multiscale elastoplastic model, based on the hierarchical organization of bone. Corresponding elasticity maps across the organ enter a Finite Element simulation of a typical load case, and the resulting stress states are increased in a proportional fashion, so as to check the safety against ultimate material failure. In the young patient investigated, even normal physiological loading is probable to already imply plastic events associated with the hydrated mineral crystals in the bone ultrastructure, while the safety factor against failure is still as high as five.

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1. INTRODUCTION

1 The broad scattering of ultimate forces bearable by human vertebrae [1–4] has motivated the quest
 2 for indicators of the actual, i.e. patient-specific, strength exhibited by a particular vertebral body
 3 of interest; and the most accepted indicator in this context remains the "bone mineral density -
 4 BMD" [5, 6]. However, it is much less clear how and to which extent BMD (alone) would govern
 5 bone fracture risk, and there is an ongoing discussion on additional or alternative risk indicators
 6 [7–12], based on quite comprehensive statistical analyses. At the same time, it is clearly felt that
 7 an engineering mechanics-based assessment of fracture risk, as it is the basis for structural design
 8 in civil and mechanical engineering, should hold the capacity to overcome the shortcomings of the
 9 purely statistics-based, population-related approaches referred to above. Accordingly, Computed
 10 Tomography- and Finite Element-based failure risk assessment of bony structures remains one of
 11 the central desires in bone biomechanics. This topic has been dealt with extensively, but broad
 12 clinical application has not been achieved yet. The reason for this could be that the physical origins
 13 of bone strength may need to be considered more deeply and rigorously than it has been attempted
 14 so far. In fact, when screening the comprehensive literature on the topic, it becomes obvious that
 15 even the most famous contributions on the topic rely on purely elastic analyses. Typically, linear
 16 regression parameters between linear elastic FE analyses and strength tests performed on the same
 17 piece of bone are established, which are typically superior to regressions between strength and
 18 some direct radiological measures [3, 13, 14]. In other studies, stress states derived from linear
 19 Finite Element analyses are averaged over suitably chosen "regions of interest", and corresponding
 20 average stress levels are thought to be relevant for fracture risk assessment [15]. Still, one might
 21 argue that the choice of such regions of interest may be quite arbitrary, but at the same time
 22 very influential on the corresponding simulation results. This would call for a deeper theoretical
 23 involvement into the material mechanics of bone and a particularly notable approach was realized
 24 for human femura [16, 17], where a certain consensus has been gained in the community as regards
 25 CT-to-mechanics calibration schemes [18–20]. Namely, elastic properties derived from CT- and
 26 mass density-based calibration schemes [21–24], are mapped onto Finite Element meshes, and for
 27 a variety of load cases, strains as obtained from an elastic simulation are assessed with respect to a
 28 failure criterion based on a constant maximum principal strain. The aforementioned consensus (or

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even enough data for potentially reaching it) is hard to find for other types of bone where only very few studies exist [25]; so that again deeper theoretical understanding is necessary, the obvious next step thereby concerning elastoplasticity. In fact, elastoplastic Finite Element simulations on bony organs have already been performed [26], and they have turned out to be useful when it comes to simulate the interaction between an implant and the neighboring bone. Still, this does not render the entire issue of bone strength assessment as being solved: There is a second challenge which needs to be overcome: The determination of relevant mechanical properties (elastic as well as elasto-plastic strength values), from clinically available CT-images. The traditional approach [15] consists of regression analyses between attenuation-related grey values and mechanical properties; however, no consensus exists on unique regression parameters; they most probably depend on various additional variables, such as CT machine settings and X-ray energies used. Still, such empirical density-stiffness relations are also used in an inverse fashion, in order to relate elastic property distributions across organs to corresponding mass densities [27]. In this case, the elastic property distributions were determined such that given physiological load cases resulted in targeted strain magnitudes as proposed in the framework of the Mechanostat theory [28]. On the other hand, a more physics-oriented approach would ask for the origin of elasticity and strength of the material found within each voxel to be found in a Computed Tomograph – this origin being the material microstructure and the mechanical properties of its elementary components, in the case of bone: hydroxyapatite, collagen, and water with some non-collageneous organics. Thanks to recent discoveries concerning universal patterns in bone tissue composition and microstructure [29–32], as well as their effects on the tissue's elasticity and strength [33–35], we are now in the position to rigorously relate tissue mass densities and porosities at different scales of observation, to corresponding elastic and strength values. Still, these mass densities need to be related to the grey values defining a CT-image, in order to obtain the desired (unique and reliable) relation between CT-numbers and mechanical properties. In this context, it proved useful to translate the chemical characteristics (and hence the mass density) of the matter found in each and every voxel making up a 3D stack of Computed Tomography images, into the corresponding X-ray attenuation coefficients, based on the publicly available NIST data base [36] and the average rule for attenuation coefficients [18,37–43]. However, such a determination of density/chemistry-attenuation relations is often challenged by the fact that the X-ray attenuation coefficients do not only depend on the material's chemistry (which establishes a link to mass density), but also on the X-ray energy used for the CT image; and as a rule, the latter is not documented in a clinical setting. Extending, modifying, and adapting recent results gained in the

context of ceramic biomaterials [44] the present paper describes, by example of a human vertebra, how to overcome this last obstacle, through combining X-ray attenuation averaging at different scales and over different tissues, with "universal" compositional characteristics of the latter. This opens the way to fully patient- and site-specific mass density and composition maps throughout the investigated organs. Using this composition as input for mathematically cast explicit quantification of the hierarchical microstructure of bone, and its effect on the unique mechanical properties of this biological material, will finally allow us to extend a safety assessment philosophy which is used very successfully in everyday steel or concrete engineering, towards the needs of a very intricate life science problem: the breaking of bones.

As regards the use of fundamental principles, X-ray physics-derived (voxel-specific) mineral, collagen, and water contents for a human vertebra will be fed into a homogenization theory-based multiscale mechanics representation of bone [34, 35], which has been extensively validated experimentally for both elastic and strength properties. This will provide calibration- and phantom-free conversion of CT data into mechanical properties.

As regards the safety assessment concept, voxel-specific elastic properties gained by means of the aforementioned conversion method will enter linear elastic Finite Element simulations, so as to deliver organ-wide stress distributions related to a typical load case. These local, i.e. element-specific, stress states are subsequently increased in a proportional fashion, so as to check which elastoplastic events would develop in the hydrated mineral crystals, before brittle fracture of molecular collagen would mark ultimate material failure. The corresponding proportionality factors quantify the element-specific safety against fracture, which is a lower bound to the overall organ fracture risk.

2. MATERIALS AND METHOD

2.1. *Conversion of CT grey values into energy-dependent attenuation coefficients, based on statistical image evaluation and on soft tissue composition rules*

A CT scan of a motion segment of a 15-years-old male patient, consisting of two lumbar vertebral bodies L3 and L4, was obtained from Mater Dei Hospital, Malta. The HiSpeed Dual medical CT scanner from General Electrics was employed in helical mode, with the following parameters: source voltage: 140 kV, source current: 110 μ A, exposure time: 1000 ms, image pixel size: 0.324 mm, and slice spacing: 1.25 mm. In the respective CT images, which capture not only the bone

structure of a vertebral body, but also the surrounding soft tissue, the X-ray attenuation information is stored in terms of 8-bit grey values, increasing with intensifying attenuation. In order to separate soft and hard tissues, all the grey values found in the 3D image are evaluated first in terms of a (normalized) histogram, representing a probability density function for the grey value of one voxel which is randomly chosen from the 3D image. More precisely, in such histogram, we identify two peaks related to the most frequently occurring grey values containing (i) adipose tissues (GV_{fat}) and (ii) inner organs (GV_{soft}), with $GV_{fat} < GV_{soft}$. In order to separate bone tissue voxels from their surroundings, we consider the first minimum value of the probability density function, which appears right of GV_{soft} , i.e. for $GV > GV_{soft}$. We denote this minimum value as segmentation threshold GV_{thr} .

In order to back-translate the user-selected grey scale values into the underlying X-ray attenuation values, we consider the linear relation between grey values and X-ray energy-dependent attenuation coefficients, involving three unknowns: two proportionality factors a and b , and the X-ray energy $\mathcal{E}$:

$$\mu(\mathcal{E}) = a(\mathcal{E}) \times GV + b(\mathcal{E}) \quad (1)$$

Eqn.(1) expresses that the X-ray attenuation coefficient is no classical material property; namely, it depends not only on the matter contained in a specific voxel, but also on the energy used for X-ray penetration of the investigated object. In order to identify the energy-dependent slope and intercept functions, $a(\mathcal{E})$ and $b(\mathcal{E})$, we specify (1) for the aforementioned two landmark values concerning fat and soft tissues:

$$\mu_{fat}(\mathcal{E}) = a(\mathcal{E}) \times GV_{fat} + b(\mathcal{E}) \quad (2)$$

$$\mu_{soft}(\mathcal{E}) = a(\mathcal{E}) \times GV_{soft} + b(\mathcal{E}) \quad (3)$$

In order to determine the functions at the left-hand side of Eqn.(2) and (3), we consider two fundamental relations:

- The mass attenuation coefficient of a chemical substance i with mass density ρ_i , $(\mu/\rho)_i$, is a function of its chemical composition, as is openly accessible from the National Institute of Standards and Technology (NIST) [36]; in particular, the mass attenuation coefficient of fat is directly given, so that the mass density of fat $\rho_{fat}=0.95 \text{ g/cm}^3$ [45] gives access to the attenuation coefficient of fat, as depicted by the black line in Figure 1.

- The X-ray attenuation coefficient of a composite material located inside one voxel is identical to the volume average of the X-ray attenuation coefficients of the material's single constituents [37–39]

$$\mu(\mathcal{E}) = \sum_{i=1}^{N_C} \mu_i(\mathcal{E}) \times f_i \quad (4)$$

with μ_i , $i = 1, \dots, N_C$, as the X-ray attenuation coefficient of constituent i , N_C as the total number of constituents, and with f_i as the volume fraction of constituent i within the considered voxel.

Figure 1. X-ray attenuation coefficients of the elementary constituents of bone tissue, and of adipose tissue; as functions of the photon energy (Data reproduced from [36], in combination with the mass densities of adipose tissue [45], of collagen [46], and of hydroxyapatite [35])

Applying Eqn.(4) to the soft tissue, i.e. inner organs and muscles localized around the spine, yields

$$\mu_{soft}(\mathcal{E}) = \mu_{H_2O}(\mathcal{E}) \times f_{H_2O} + \mu_{org}(\mathcal{E}) \times (1 - f_{H_2O}) \quad (5)$$

where the attenuation coefficient of water follows from its chemical formula H_2O , see second-lowest line in Fig.1, and that of organic material is approximated by the smallest amino acid, glycine (making up a high percentage of collagen, and being similar in structure to the remaining portion of collagen [47]), with chemical formula $C_2H_5NO_2$, see dotted line in Fig.1. The volume fraction of water, f_{H_2O} , is accessed through the average rule for the soft tissue mass density,

$$\rho_{soft} = f_{H_2O} \times \rho_{H_2O} + (1 - f_{H_2O}) \times \rho_{col} \quad (6)$$

namely through

$$f_{H_2O} = \frac{\rho_{soft} - \rho_{col}}{\rho_{H_2O} - \rho_{col}} \quad (7)$$

with the soft tissue mass density of tissues around the spine being documented as $\rho_{soft}=1.052 \text{ g/cm}^3$ [45] and $\rho_{col}=1.42 \text{ g/cm}^3$ [46].

Conclusively, we solve Eqn.(2) and (3), while considering Eqn.(4)-(7), for $a(\mathcal{E})$ and $b(\mathcal{E})$.

2.2. Identification of X-ray photon energy and of extracellular bone tissue mass density, based on general bone tissue composition rules

As supplementary voxels of interest, we consider the densest voxel in the *corpus vertebrae*, GV_{max} , which hosts part of the "cortical shell" of the vertebra. However, as seen from the microCT image of Figure 2, this shell is very thin, with a typical thickness of only 230 microns [48–50], so that the densest voxel (with a voxel size $l_{voxel}=324$ microns) does not only contain cortical bone, but also adjacent vascular porosity or soft tissue around. Hence, we consider one additional voxel, namely the densest neighbor of the voxel exhibiting GV_{max} , also containing both extravascular tissue resembling "cortical bone", as well as vascular porosity or soft tissue, with GV_{soft} , see Figure 3. The grey values related to the aforementioned two voxels are used for identification of the grey

Figure 2. MicroCT image of vertebra, adapted from Malandrino et al (2012) [51]; showing very thin "cortical shell", as well as original position and orientation of cubic samples cut from the investigated mid-height vertebra.

value GV_{ev} which is related to a (fictitious) voxel being filled by extravascular bone tissue only. The aforementioned cortical shell thickness either lies fully within one single voxel, or it contributes to the attenuation coefficients of two neighboring voxels. In the latter case, the cortical shell thickness is partitioned between the two neighboring voxels, with voxel-specific parts l_1 and l_2 , $l_1 + l_2 = l_{cort}$, see Figure 3; and the former case can be seen as a limit of the latter, characterized by $l_2 = 0$ and $l_1 = l_{cort}$. The area of the voxel not covered by the cortical shell is considered as surrounding soft tissue on the exterior side of the vertebra, and gel-type, watery fluid- and soft cells-filled vascular pore space on the interior side. Since both materials exhibit very similar attenuation properties, we consider the same grey value for water and soft tissue.

Figure 3. Partition of cortical shell (with thickness l_{cort}) between two neighboring voxels (with edge length l_{voxel})

In order to use these partitioning considerations for identification of the *a priori* unknown grey value for the extravascular tissue, GV_{ev} , we use grey value-to-attenuation relation (1) in average rule (4), so as to show the existence of an average rule for the grey values as well,

$$GV = \sum_{i=1}^{N_C} GV_i \times f_i \quad (8)$$

When applying (8) to the cortical shell partitioned between two voxels as depicted in Figure 3, one arrives at the following system of equations:

$$\frac{l_1}{l_{voxel}} \times GV_{ev} + \left(1 - \frac{l_1}{l_{voxel}}\right) \times GV_{soft} = GV_{max} \quad (9)$$

$$\frac{l_2}{l_{voxel}} \times GV_{ev} + \left(1 - \frac{l_2}{l_{voxel}}\right) \times GV_{soft} = GV_{max-1} \quad (10)$$

$$l_1 + l_2 = l_{cort} \quad (11)$$

Solving these three equations for the unknowns GV_{ev} , l_1 , and l_2 , yields in particular the sought value for GV_{ev} as function of all the already identified grey values and length dimensions,

$$GV_{ev} = \frac{l_{voxel}}{l_{cort}} \times (GV_{max} + GV_{max-1} - 2GV_{soft}) + GV_{soft} \quad (12)$$

In order to identify the X-ray energy $\mathcal{E}$, which then will allow for a unique scaling relation between grey values and attenuation coefficients, as given through Eqn.(1), we consider two independent ways to access the attenuation coefficient of the extravascular bone material, μ_{ev} . These two ways need to deliver *one single, unique result*:

- The first access to μ_{ev} is provided through

$$\mu_{ev}(\mathcal{E}) = a(\mathcal{E}) \times GV_{ev} + b(\mathcal{E}) \quad (13)$$

fed with the energy-dependent proportionality constants derived from Eqn.(1)-(7);

- The second access is provided through repeated use of Eqn.(4) for upscaling attenuation coefficients from the level of the elementary constituents of bone tissue, up to the level of the extravascular bone material.

As regards the latter item, the NIST-data base allows for retrieval of the attenuation coefficients of the (mechanically relevant) elementary constituents of extracellular bone tissue: hydroxyapatite, organic material, and water. The two latter ones have been already introduced in Section 2.1, and the chemical formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ allows for obtaining the hydroxyapatite-related attenuation curve, as depicted in Fig.1. Use of these functions in Eqn.(4), i.e. upscaling from the elementary to the extracellular level, yields

$$\mu_{ec}^{up}(\mathcal{E}) = \mu_{HA}(\mathcal{E}) \times f_{HA}^{ec} + \mu_{org}(\mathcal{E}) \times f_{org}^{ec} + \mu_{H_2O}(\mathcal{E}) \times f_{H_2O}^{ec} \quad (14)$$

with $f_{H_2O}^{ec} + f_{org}^{ec} + f_{HA}^{ec} = 1$

The constituent volume fractions f_{HA}^{ec} , f_{org}^{ec} , and $f_{H_2O}^{ec}$ (measured per volume of extracellular (*ec*) material) depend in a unique fashion on the tissue's extracellular mass density ρ_{ec} , see Fig.4; as evidenced in [30], from a multitude of weighing tests on dried, demineralized, deorganified, and ashed samples collected over 80 years of research [46, 52–62]. This implies the existence of a function $\mu_{ec}^{up}(\mathcal{E}, \rho_{ec})$, which can then be upscaled to the extravascular level through

$$\mu_{ev}^{up}(\mathcal{E}, \rho_{ec}) = \phi_{lac} \times \mu_{H_2O}(\mathcal{E}) + (1 - \phi_{lac}) \times \mu_{ec}^{up}(\mathcal{E}) \quad (15)$$

with as the lacunar porosity, $\phi_{lac}=0.10$ [35, 63]. Expressions (13) and (15) need to deliver the same

Figure 4. Universal composition rules in bone tissue [30]: bilinear relations between constituent volume fractions and tissue mass density

result, which we express as a ratio being equal to one,

$$\mu_{ev}(\mathcal{E})/\mu_{ev}^{up}(\mathcal{E}, \rho_{ec}) = R(\mathcal{E}, \rho_{ec}) = 1 \quad (16)$$

Equation (16) defines a non-bijective function of the mass density as a function of the energy, i.e. one energy value, more than one energy value, or no energy value, may be related to one and the same mass density value. From this function, the value for the extracellular mass density, which is related to only one, i.e. unique, energy value, will be identified: this provides access to both the X-ray energy used for the image, and to the extracellular mass density of the investigated bone tissue from a human vertebra.

2.3. Vertebra tissue mass density from weighting tests

In order to check the relevance of the value for the tissue mass density retrieved by means of the strategy outlined in Section 2.2, an independent experimental route towards ρ_{ec} is pursued, through the following protocol: From the mid-height portion of an elderly human vertebral body provided by the Medical University of Vienna, a slice was cut out by means of a band saw under continuous water irrigation (300Cp, Exakt GmbH, Germany). Out of this slice, eight cubes with 5-6 mm edge length were extracted by means of a wheel saw (Isomet, Buehler, USA). All the specimens had a face containing the external vertebral cortex, as can be seen in Fig.2. Two exactly parallel opposite surfaces orthogonal to the cortex layer were cut, whereby attention was paid on maintaining the cortex undamaged. Then, the specimens were immersed in an ultrasonic bath with 0.9% saline solution and standard soap, for 3 minutes at 40°C, in order to allow for mixing of the solution with the soap. Afterwards, the specimens were left during 24 hours at the same temperature for lipid dissolution. They were then washed with 0.9% saline solution for 10 minutes at 40°C under ultrasonic bath, and finally under flushing water at room temperature, in order to further clean the pores. The extracellular tissue density was measured by means of Archimedes' principle: The weight W_s of each specimen completely submerged in distilled water (air bubbles were removed under vacuum) was recorded by means of an analytical balance (PG403-S, Mettler-Toledo GmbH, Switzerland), as was the weight in air, W_a , the latter being accessible after centrifugation at 400 g for 15 minutes, in order to remove water from the vascular and lacunar pores. The mass density ρ_{ec} of the extracellular bone matrix was then determined according to:

$$\rho_{ec} = \frac{W_a}{W_a - W_s} \quad (17)$$

2.4. Voxel-specific tissue composition

Once the photon energy $\mathcal{E}$ is known, it gives access to the extravascular bone matrix attenuation coefficient via Eqn.(15), which can then be upscaled to the macroscopic bone material level: Therefore, we specify the average rule (4) for the constituents "extravascular bone matrix" [with volume fraction $(1-\phi_{vas})$] and vascular porosity (with volume fraction ϕ_{vas}), yielding

$$\mu_{macro} = \phi_{vas} \times \mu_{H_2O} + (1 - \phi_{vas}) \times \mu_{ev} \quad (18)$$

206 Solving this equation for ϕ_{vas} gives access to voxel-specific values for the vascular porosity,

$$\phi_{vas} = \frac{\mu_{macro} - \mu_{ev}}{\mu_{H_2O} - \mu_{ev}} \quad (19)$$

207 ϕ_{vas} can be also used to provide voxel-specific mass densities at the macroscopic scale

$$\rho_{macro} = \phi_{vas} \times \rho_{H_2O} + (1 - \phi_{vas}) \times \rho_{ev} \quad (20)$$

208 based on mass densities at the extravascular scale

$$\rho_{ev} = \phi_{lac} \times \rho_{H_2O} + (1 - \phi_{lac}) \times \rho_{ec} \quad (21)$$

209 Moreover, we are also interested in the apparent mass densities of mineral and collagen, i.e.
 210 the "bone mineral and collagen mass densities", the former one being sometimes referred to as
 211 vBMD [64].

212 Therefore, we convert constituent volume fractions into apparent mass densities of
 213 hydroxyapatite, collagen, and water and non collagenous proteins,

$$\rho_{HA}^{*,ec} = f_{HA}^{ec} \times \rho_{HA} \quad (22)$$

$$\rho_{col}^{*,ec} = 0.9 \times f_{org}^{ec} \times \rho_{col} \quad (23)$$

$$\rho_{H_2O+ncp}^{*,ec} = \rho_{ec} - \rho_{HA}^{*,ec} - \rho_{col}^{*,ec} \quad (24)$$

214 where we consider that 90% of the organic matter in extracellular bone matrix is collagen [65].
 215 The apparent mass densities (22) - (24) are upscaled from the extracellular to the macroscopic level
 216 through

$$\begin{aligned} \rho_{HA}^* &= \rho_{HA}^{*,ec} \times (1 - \phi_{lac}) \times (1 - \phi_{vas}) \\ \rho_{col}^* &= \rho_{col}^{*,ec} \times (1 - \phi_{lac}) \times (1 - \phi_{vas}) \\ \rho_{H_2O+ncp}^* &= \rho_{macro} - \rho_{col}^* - \rho_{HA}^* \end{aligned} \quad (25)$$

217 2.5. Intravoxel tissue elasticity

218 The ultrastructural mass density and the spatial distribution of vascular porosities determined from
 219 a CT scan of a patient by means of a series of X-ray physics considerations, as described in Section

2.4 (see in particular Figure 11), are now converted into voxel-specific elastic properties, on the basis of the multiscale homogenization scheme depicted in Figure 5.

Figure 5. Multiscale micromechanical representation of bone material, according to Morin and Hellmich [35]

This scheme, described in great detail in [29,33,35,66], quantifies how the elementary mechanical constituents of bone, namely hydroxyapatite, collagen, and water with some non-collagenous organics, as well as their dosages within the extracellular bone matrix, determine the elastic properties of bone at different length scales. It has been extensively validated experimentally, through an ultrasound data base stemming from a wealth of bone sources - equine cortical bone [67], bovine tibia [52], drug-treated rabbit bone [68], whale malleus, incus, stapes, periotic and t. bulla [53], fin whale t. bulla [69], dugong rib, elephant radius, human femur, deer antler [54] - in combination with "universal" composition and mineralization rules for bone tissues [30–32, 70]. The latter were derived from a wealth of biophysical, biochemical, and biomechanical experimental sources [52, 55, 57–60, 71–79]. In this context, bone is represented by a series of representative volume elements, the size of which is significantly larger than that of the inhomogeneities found within such a volume [see e.g. Figure 5(f) with the vascular pores' diameters being scale-separated from the millimeter-size of the cortical or trabecular RVE]. Also, representation of the microstructure within the RVE is reduced to the bare minimum needed for elasticity

homogenization: the domain within the RVE is subdivided into the minimum number of material phases with distinctively differing physical properties: their volume fractions [such as the vascular porosity in Figure 5(f)], their (average) elastic properties [such as those of the vascular pores and of the extravascular bone matrix in Figure 5(f)], the most fundamental shape characteristics (cylinders representing vascular pores in the case of cortical/trabecular bone), and their interaction [pore inclusions embedded into a solid matrix in Figure 5(f)]. This representation implies the following Mori-Tanaka-scheme-type expression for the stiffness of cortical/trabecular bone [37],

$$\mathbb{C}_{macro} = \left\{ \phi_{vas} \mathbb{C}_{H_2O} : [\mathbb{I} + \mathbb{P}_{cyl} : (\mathbb{C}_{H_2O} - \mathbb{C}_{ev})]^{-1} + (1 - \phi_{vas}) \mathbb{C}_{ev} \right\} : \left\{ \phi_{vas} [\mathbb{I} + \mathbb{P}_{cyl} : (\mathbb{C}_{H_2O} - \mathbb{C}_{ev})]^{-1} + (1 - \phi_{vas}) \mathbb{I} \right\}^{-1} \quad (26)$$

with ϕ_{vas} as the vascular porosity, $\mathbb{C}_{ev}$ and $\mathbb{C}_{H_2O}$ as the elastic stiffness of the extravascular bone matrix and water, $\mathbb{I}$ as the fourth-order identity tensor with components $I_{ijkl} = \frac{1}{2}(\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})$, with the Kronecker delta δ_{ij} being equal to 1 for $i = j$ and zero otherwise; and $\mathbb{P}_{cyl}$ as the fourth-order Hill tensor accounting for the cylindrical shape of the inclusions embedded into a transversely isotropic matrix [66].

Within the investigated vertebral body, the vascular porosity is varying from voxel to voxel, as depicted in Figure 11; all other quantities in Eqn.(26) are constant. Particularly, the extravascular stiffness follows from feeding the homogenization scheme of Figure 5 with an ultrastructural mass density resulting from Eq. (16) (and corresponding ultrastructural volume fractions of mineral, collagen, and water as given in Figure 12) and with a lacunar porosity of 10 % [35,63].

The stiffness tensor of water reads as $\mathbb{C}_{H_2O} = 3\mathbb{I}_{vol}k_{H_2O}$, with the bulk modulus of water amounting to $k_{H_2O}=2.3$ GPa [80], and with $\mathbb{I}_{vol}$ as the volumetric part of the fourth-order identity tensor, with components $I_{vol,ijkl} = \frac{1}{3}\delta_{ij}\delta_{kl}$.

2.6. Linear Finite Element simulations

The used Finite Element model is based on earlier work published in [81], see Figure 6: It consists of 125253 solid elements accounting for the trabecular bone tissue, and 17185 shell elements accounting for the cortical bone tissue. For the present study, we realized a cortical shell thickness of $l_{cort}=0.23$ mm [48–50]; while the caudal and cranial (bony) endplates were assigned thicknesses of $l_{end} = 1$ mm [82, 83]. In order to investigate the effect of heterogeneity at the finite element level (i.e. with gradients measured along a few millimeters), we considered two ways of assigning material properties to the Finite Element model of Figure 6:

Figure 6. Finite Element mesh of the patient-specific vertebral body

- For the so-called "homogeneous model", the entire trabecular bone compartment is assigned the same elastic stiffness tensor, namely that related to the average vascular porosity found in that compartment, $\phi_{vas}^{trab}=0.86$. More precisely, given the "short-beam"-type nature of our structure, we assign the longitudinal Young's modulus

$$E_{macro,3} = \frac{1}{C_{macro,3333}^{-1}} = 1322 \text{ MPa} \quad (27)$$

with $C_{macro,3333}^{-1}$ as the longitudinal normal component of the compliance tensor $\mathbb{C}_{macro}^{-1}$ and the longitudinal Poisson's ratio

$$\nu_{31} = -C_{macro,1133}^{-1} \times E_{macro,3} = 0.19 \quad (28)$$

as "formally isotropic" material properties to the FE model, as is normally considered as an appropriate approximation in bone organ mechanics [43, 84, 85]. As regards the very thin cortical shell around the organ, whose thickness of 230 microns does not quite allow for accommodation of Haversian systems (and hence of the vascular porosity), we adopt the "macroscopic" stiffness tensor $\mathbb{C}_{macro}$ related to zero-porosity, i.e. that of the extravascular bone matrix according to Eqn.(48). More precisely, as before, we assign as formally isotropic properties, a Young's modulus and a Poisson's ratio of 9406 MPa and of 0.28, respectively, to the shell finite elements.

- For the "heterogeneous model", the cortical shell treatment does not differ, while the element-specific stiffness tensors are computed from the vascular porosity values assigned to the centroids of the finite elements. The interpolation between the CT data and the Finite Elements is made by finding the three CT pixels closest to the finite element centroid, and by assigning

the porosity values to the element as if its centroid would be the barycentre of these three points.

Since the focus of the present study is on the effect of material properties rather than on different loading conditions, we consider a very basic load case, related to a "static deadload": 1 MPa pressure is applied to the cranial endplate, while the caudal endplate is pinned in all space directions. The pressure magnitude is indeed related to "quasi-physiological conditions": half of the body weight of a person of 75 kg mass in the gravitational field of the earth would results in a pressure of 0.75 MPa, when evenly distributed across the surface area $A_{end}=869 \text{ mm}^2$ of the cranial endplate.

In order to test the robustness of our model, we compare the computational results of four models with increasing element sizes, i.e. decreasing amount of elements, namely 229138, 143159, 105763, and 82848 elements. More precisely, we compute, for all element sizes, the strain energy density in one element n as:

$$\psi_n = \frac{1}{2} \varepsilon_n : \mathbb{C}_{macro,n} : \varepsilon_n \quad (29)$$

with ε_n denoting the macroscopic strains in element n ; so that the average strain energy over the entire organ reads as:

$$\bar{\psi}_{organ} = \frac{1}{V_{organ}} \sum_{n=1}^{N_n} \psi_n \times V_n \quad (30)$$

with N_n as the number of finite elements, with V_n as the volume of the n -th finite element and V_{organ} as the volume of the entire organ. The results of the linear elastic Finite Element simulations are then used for safety assessment of the investigated structure. More precisely, we check by which proportionality factor the determined element-specific macroscopic stresses needed to be increased, so as to reach ultimate macroscopic bone material failure. This stress increase, however, is subjected to macroscopic bone RVEs in an incremental fashion, allowing for elastoplastic deformation states prior to the ultimate material failure (which obviously depends on these elastoplastic deformations). In order to quantify them, we extend the hierarchical scheme depicted in Figure 5 to the realm of elastoplasticity, extending earlier work published in [34]. There, the liquid crystal-type water interfaces between the mineral crystals and/or crystal clusters in the extrafibrillar space [see Figure 5(c)], have been identified as the major nanoscopic origin of bone elastoplasticity, both from the high interaction energies between water and hydroxyapatite as evidenced by several molecular dynamics and nuclear magnetic resonance studies [86–90], and more importantly, by successfully predicting, based on experimentally obtained upscaling mineral and collagen strengths [91–93], the macroscopic strengths of different types of bone [94–111]. Such successful representations of

bone microstructure for mechanical property predictions, which do not resolve the material up to the highest resolution ever possible, but rather focus on reliable consideration of the most important micromorphological features, have not only been developed in the framework of continuum micromechanics or random homogenization theory, but also in alternative theoretical frames, such as that of lattice models [112].

Thereby, the fluid-induced, mutual sliding of crystals is associated to elastoplastic hydroxyapatite mineral phases, which are oriented uniformly in space directions given through Eulerian angles ϕ and θ related to spherical coordinates. These phases obey the following constitutive equations: The needle-shaped mineral phase oriented in space direction (ϕ, θ) follows an elasto-plastic stress-strain relation,

$$\boldsymbol{\sigma}_{HA,\phi\theta} = \mathbb{C}_{HA} : (\boldsymbol{\varepsilon}_{HA,\phi\theta} - \boldsymbol{\varepsilon}_{HA,\phi\theta}^p) \quad (31)$$

with $\mathbb{C}_{HA} = 3k_{HA}\mathbb{I}_{vol} + 2G_{HA}\mathbb{I}_{dev}$ as the isotropic elasticity of hydroxyapatite, $k_{HA}=82.6$ GPa and $G_{HA}=44.9$ GPa [113], $\mathbb{I}_{dev} = \mathbb{I} - \mathbb{I}_{vol}$ as the deviatoric component of the fourth-order identity tensor, $\boldsymbol{\varepsilon}_{HA,\phi\theta}$ and $\boldsymbol{\varepsilon}_{HA,\phi\theta}^p$ as the (average) total and plastic strains in the $(\phi\theta)$ -oriented needle-shaped phase, and $\boldsymbol{\sigma}_{HA,\phi\theta}$ as the corresponding average stress. Plastic events are quantified by means of a Mohr-Coulomb criterion,

$$\mathcal{F}_{HA,\phi\theta} = \mathcal{F}(\boldsymbol{\sigma}_{HA,\phi\theta}) = \beta\sigma_{HA,\phi\theta}^I - \sigma_{HA,\phi\theta}^{III} - \sigma_{HA}^y \leq 0 \quad (32)$$

with β as the ratio between the compressive and the tensile yield stresses ($\beta = 12$), σ_{HA}^y as the compressive yield stress ($\sigma_{HA}^y=570$ MPa), and $\sigma_{HA,\phi\theta}^I \geq \sigma_{HA,\phi\theta}^{II} \geq \sigma_{HA,\phi\theta}^{III}$ as the (sorted) principal stresses in the $(\phi\theta)$ -oriented mineral phase. The strength parameters follow from tests on porous hydroxyapatite polycrystals [114–122], mimicking those occurring in the extrafibrillar space of Figure 5(c). Potential occurrence of plastic events follows the Kuhn-Tucker conditions [123] (actually proposed already in 1938 by Melan [124])

$$\dot{\lambda}_{HA,\phi\theta} \geq 0, \quad \mathcal{F}_{HA,\phi\theta} \leq 0 \quad \text{and} \quad \dot{\lambda}_{HA,\phi\theta} \times \mathcal{F}_{HA,\phi\theta} = 0 \quad (33)$$

in conjunction with the following, non-associative, isochoric flow rule:

$$\dot{\boldsymbol{\varepsilon}}_{HA,\phi\theta}^p = \dot{\lambda}_{HA} \frac{\partial \mathcal{G}}{\partial \boldsymbol{\sigma}_{HA,\phi\theta}} \quad \text{with} \quad \mathcal{G}(\boldsymbol{\sigma}_{HA,\phi\theta}) = \sigma_{HA,\phi\theta}^I - \sigma_{HA,\phi\theta}^{III} \quad (34)$$

whereby the dot denotes the time derivative, $\mathcal{G}$ denotes the flow potential, the derivative of which accounts for the plastic flow direction, and $\dot{\lambda}_{HA,\phi\theta}$ denotes the plastic multiplier quantifying the amount of plastic strain.

Such plastic events imply more and more micro-stresses to be transferred to the molecular collagen of Figure 5(a), which finally fails in a brittle manner, according to a Rankine-type criterion,

$$\mathcal{F}_{col} = \max_{i \in \{I, II, III\}} |\sigma_{col}^i| - \sigma_{col}^{ult} \leq 0 \quad (35)$$

with σ_{col}^i , $i = I \dots III$, as the eigenstresses in the molecular collagen assembly at the wet collagen (microfibrillar) scale, and σ_{col}^{ult} as the strength of molecular collagen. The latter quantity is derived from experiments on rat tail tendon, which, under wet conditions, exhibits a strength of 106.1 MPa [93]. We consider close packing of collagen, so as to get access to the properties of molecular collagen. It is known from neutron diffraction studies [46, 75] that diffractive spacing (a measure for the lateral distance of collagen molecules) reduces from 1.52 nm (for wet collagen) to 1.09 nm [for maximally packed (dry) collagen]. Accordingly, the cross sectional area of a tensile specimen would reduce by the ratio $(1.52/1.09)^2$, so that the strength of molecular collagen follows to be $(1.52/1.09)^2$ times higher than that of wet collagen, i.e. 206 MPa.

These elastoplastic and failure laws need to be homogenized over the RVEs depicted in Figure 5, so as to determine the elastoplastic behavior of a piece of cortical/tabecular bone illustrated in Figure 5(f). This is achieved in the framework of continuum micromechanics [125] by means of stress and strain averaging rules [126, 127] and concentration-influence relations [128]; applied to each of the RVEs depicted in Figure 5. As regards the extrafibrillar RVE, homogeneous strains $\mathbf{E}_{ef}$ are prescribed in terms of displacements

$$\boldsymbol{\xi}(\mathbf{x}) = \mathbf{E}_{ef} \cdot \mathbf{x} \quad (36)$$

at the boundary of the RVE - $\mathbf{x}$ is the "microscopic" location vector with a resolution much smaller than the RVE. This boundary condition implies that kinematically compatible microstrains $\boldsymbol{\varepsilon}(\mathbf{x})$

$$\boldsymbol{\varepsilon}(\mathbf{x}) = \nabla^s \boldsymbol{\xi}(\mathbf{x}) \quad (37)$$

fulfill the following strain average rule [114, 125]

$$\mathbf{E}_{ef} = f_{HA}^{ef} \int_{\phi=0}^{2\pi} \int_{\theta=0}^{\pi} \boldsymbol{\varepsilon}_{HA,\phi\theta} \frac{\sin \theta}{4\pi} d\phi d\theta + (1 - f_{HA}^{ef}) \boldsymbol{\varepsilon}_{ic} \quad (38)$$

with f_{HA}^{ef} as the volume fraction of hydroxyapatite within the extrafibrillar RVE, and $\boldsymbol{\varepsilon}_{ic}$ as the (average) strains in the inter-crystalline space. Moreover, when applying the principle of virtual powers to this RVE, resulting in the so-called Hill's lemma [125], one arrives at an additional stress average rule for macroscopic stresses acting on the extrafibrillar RVE,

$$\boldsymbol{\Sigma}_{ef} = f_{HA}^{ef} \int_{\phi} \int_{\theta} \boldsymbol{\sigma}_{HA,\phi\theta} \frac{\sin \theta}{4\pi} d\phi d\theta + (1 - f_{HA}^{ef}) \boldsymbol{\sigma}_{ic} \quad (39)$$

with the microstresses $\boldsymbol{\sigma}(\mathbf{x})$ fulfilling the equilibrium condition

$$\text{div } \boldsymbol{\sigma}(\mathbf{x}) = 0 \quad (40)$$

Linearity of Eqn.(40), (37), (36), and (31) implies the strains in the $(\phi\theta)$ -oriented mineral phase to depend multi-linearly on the homogeneous strains $\mathbf{E}_{ef}$ and on the plastic (micro-)strains $\boldsymbol{\varepsilon}_{HA,\phi\theta}^p$ found in all mineral phases,

$$\boldsymbol{\varepsilon}_{HA,\phi\theta} = \mathbb{A}_{HA,\phi\theta} : \mathbf{E}_{ef} + \int_{\Phi} \int_{\Theta} \mathbb{D}_{\phi\theta\Phi\Theta} : \boldsymbol{\varepsilon}_{HA,\Phi\Theta}^p \frac{\sin \Theta}{4\pi} d\Phi d\Theta \quad (41)$$

Similarly, the microstrains $\boldsymbol{\varepsilon}_{ic}$ found in the inter-crystalline pores read as

$$\boldsymbol{\varepsilon}_{ic} = \mathbb{A}_{ic} : \mathbf{E}_{ef} + \int_{\Phi} \int_{\Theta} \mathbb{D}_{ic,\Phi\Theta} : \boldsymbol{\varepsilon}_{HA,\Phi\Theta}^p \frac{\sin \Theta}{4\pi} d\Phi d\Theta \quad (42)$$

with $\mathbb{A}_{HA,\phi\theta}$ and $\mathbb{A}_{ic}$ as the concentration tensors of the hydroxyapatite phase oriented in direction $(\phi\theta)$ and of the inter-crystalline phase respectively, and $\mathbb{D}_{\phi\theta\Phi\Theta}$ and $\mathbb{D}_{ic,\Phi\Theta}$ accounting for the influence of plastic strains occurring in the $(\Phi\Theta)$ -oriented phase, on the total strains occurring in the mineral phase oriented in $(\phi\theta)$ -direction and in the inter-crystalline phase, respectively. These concentration and influence tensors are derived from extended "eigenstressed" Eshelby problems [125]; as given in more detail in [129, 130]. Finally, repeated use of Hill's lemma yields the upscaled "macroscopic" elastoplastic constitutive equation as

$$\boldsymbol{\Sigma}_{ef} = \mathbb{C}_{ef} : (\mathbf{E}_{ef} - \mathbf{E}_{ef}^p) \quad (43)$$

where $\mathbb{C}_{ef}$ is the homogenized stiffness tensor of the extrafibrillar space, reading as

$$\mathbb{C}_{ef} = f_{HA}^{ef} \mathbb{C}_{HA} : \int_{\theta} \int_{\phi} \mathbb{A}_{HA,\phi\theta} \frac{\sin \theta}{4\pi} d\phi d\theta + (1 - f_{HA}^{ef}) \mathbb{C}_{H_2O} : \mathbb{A}_{ic} \quad (44)$$

and the extrafibrillar plastic strains $\mathbf{E}_{ef}^p$ fulfill

$$\mathbf{E}_{ef}^p = \mathbb{C}_{ef}^{-1} : f_{HA}^{ef} \int_{\theta} \int_{\phi} {}^t \mathbb{A}_{HA,\phi\theta} : \mathbb{C}_{HA} : \boldsymbol{\varepsilon}_{HA,\phi\theta}^p \frac{\sin \theta}{4\pi} d\phi d\theta \quad (45)$$

whereby the superscript t denotes the transpose of the concentration tensor (${}^t A_{ijkl} = A_{klij}$). Equations (31–45) allow one to determine the plastic events in the crystal phases caused by extrafibrillar loading - once Eqn.(38), (39), (41), (42), (44), (45) are discretized in space, here by means of the numerical scheme proposed in [131], and Eqn.(33) and (34) are discretized in (chronological) time, here by means of the return mapping algorithm [123, 132]. In particular, the multisurface plasticity concept is realized for Mohr-Coulomb plasticity [133]. This is done for all RVEs depicted in Figure 5, so as to relate macroscopic loading at the level of cortical/trabecular bone, to plastic strains in the crystalline, extrafibrillar, extracellular, and extravascular phases in Figure 5, until the molecular collagen stresses fulfill failure criterion (35).

Within this theoretical framework, two types of "safety factors", "loading degrees", or "distances to failure" are determined:

- The macroscopic, trabecular bone-related stresses $\boldsymbol{\Sigma}_{macro}$ determined in each finite element are first multiplied with a factor χ_y related to just reaching the yield limit defined by equality in Eqn.(32);

$$\boldsymbol{\Sigma}_{macro,y} = \chi_y \boldsymbol{\Sigma}_{macro} \quad (46)$$

This factor is called the yield safety factor; it is larger than one for elastic deformation states, and reduces to one once yielding starts. Its inverse is the "yield loading degree" or "distance to yielding", being smaller than one for elastic deformation states, and increasing towards one when approaching yielding states.

- Secondly, the aforementioned element-specific macroscopic stresses are multiplied with a factor χ_{ult} related to reaching the ultimate load defined by equality in Eqn.(35);

$$\boldsymbol{\Sigma}_{macro,ult} = \chi_{ult} \boldsymbol{\Sigma}_{macro} \quad (47)$$

This factor is called the (local, material-based) ultimate safety factor; it is larger than one for deformation states which the macroscopic bone material can still withstand, and reduces to one once the material fails (through collagen tearing). Its inverse is the "ultimate loading degree" or "distance to failure", being smaller than one for bearable deformation states, and increasing towards one when approaching material failure.

3. RESULTS

Evaluation of all grey values found in the investigated clinical CT image in form of the normalized histogram or probability density function depicted in Figure 7 allows for identification of the anatomical landmark values related to adipose tissue, as $GV_{fat} = 72$, to soft tissues from inner organs, as $GV_{soft} = 84$, and to bone tissue, as $GV_{bone} = 101$, see Figure 7.

In addition, the histogram provides the segmentation threshold as $GV_{thr} = 93$. From the landmark

Figure 7. Frequency distribution plot of the attenuation information in terms of grey values of the vertebral body L3

grey values for the fat and soft tissues, Eq. (1) to (7) allow for identification of the energy-dependent slope and intercept parameters a and b , see Figure 8. They allow for (still X-ray energy-dependent) conversion of grey values to the actual physical quantities they represent.

As illustrated in Figure 9, the maximum grey value occurring in the *corpus vertebrae* amounts to $GV_{max} = 164$, and its densest neighbor exhibits a grey value of $GV_{max-1} = 156$. According to Eqn.(12), this allows for identification of the grey value related to extravascular bone as $GV_{ev} = 298$. A unique relation between grey values and X-ray attenuation coefficients can be only obtained for an extracellular mass density of 1.67 g/cm^3 , being assigned to an X-ray energy of 24 keV, as is evident

Figure 8. Slope and intercept parameters a and b , as functions of the photon energy $\mathcal{E}$

Figure 9. Grey value distribution throughout vertebral cross section where the densest voxel (with GV_{max}) and its neighbor (with GV_{max-1}) occur

413 from Fig. 10, drawn on the basis of Eqn. (13) to (16). This mass density agrees very well with
 414 the one measured by Archimedes' principle as determined in Section 2.3; the latter amounting to
 415 $1.71 \pm 0.11 \text{ g/cm}^3$ (mean value $\pm$ standard deviation). Use of $\rho_{ec} = 1.67 \text{ g/cm}^3$, and of corresponding

ultrastructural volume fractions of hydroxyapatite $f_{HA}=0.27$, organic matter $f_{org}=0.29$, and water $f_{H_2O}=0.44$ in the homogenization scheme of Figure 5 yields to the extravascular stiffness tensor

$$\mathbb{C}_{ev} = \begin{pmatrix} C_{1111} & C_{1122} & C_{1133} & 0 & 0 & 0 \\ C_{1122} & C_{2222} & C_{2233} & 0 & 0 & 0 \\ C_{1133} & C_{2233} & C_{3333} & 0 & 0 & 0 \\ 0 & 0 & 0 & 2C_{2323} & 0 & 0 \\ 0 & 0 & 0 & 0 & 2C_{1313} & 0 \\ 0 & 0 & 0 & 0 & 0 & 2C_{1212} \end{pmatrix} = \begin{pmatrix} 10.56 & 4.82 & 5.30 & 0 & 0 & 0 \\ 4.82 & 10.56 & 5.30 & 0 & 0 & 0 \\ 5.30 & 5.30 & 13.06 & 0 & 0 & 0 \\ 0 & 0 & 0 & 5.99 & 0 & 0 \\ 0 & 0 & 0 & 0 & 5.99 & 0 \\ 0 & 0 & 0 & 0 & 0 & 5.75 \end{pmatrix} \text{ in GPa} \quad (48)$$

Figure 10. Extracellular mass density as function of photon energy, according to Eqn. (1)-(20), with $a(\mathcal{E})$ and $b(\mathcal{E})$ as depicted in Figure 8

Evaluation of the energy-dependent functions of Fig. 8 for the photon energy as 24 keV yields the grey value-to-attenuation conversion factors a and b as $a=0.0107 \text{ cm}^{-1}$ and $b=-0.4154 \text{ cm}^{-1}$.

420 Evaluation of relations (20) and (25) for the grey value of each and every voxel of the scanned object
 421 yields density and hydroxyapatite/collagen/water content maps throughout the vertebral body, see
 Figure 11 and 12, as well as frequency plots of the aforementioned quantities, see Figure 13. The

Figure 11. Property maps in CT slices in the transverse plane: (a) X-ray attenuation coefficient in terms of grey value, (b) vascular porosity, (c) macroscopic mass density; (d) locations of the mapped cross sections; (e) mean values of the previous quantities

422 expected (i.e. most frequently occurring) values for macroscopic mass density, mineral and collagen
 423 content amount to $\rho_{macro}^{EV}=1.04$ g/cm³, $\rho_{HA}^{*,EV}=54.1$ mg/cm³, and $\rho_{col}^{*,EV}=39.1$ mg/cm³, while their
 424 averages over the entire vertebral body amount to $\overline{\rho_{macro}}=1.07$ g/cm³, $\overline{\rho_{HA}^*}=87.8$ mg/cm³, and
 425 $\overline{\rho_{col}^*}=39.2$ mg/cm³.
 426

Figure 12. Property maps in CT slice in the transverse plane: (a) Mineral content, (b) collagen content, (c) water and non-collagenous proteins (ncp) content; (d) locations of the mapped cross sections; (e) mean values of the previous quantities

The micromechanics-based porosity-stiffness relations based on the hierarchical representation depicted in Figure 5, see also Eqn.(26) and (48), in combination with the X-ray physics-based grey value-to-porosity conversion scheme described in Section 2.4, yields organ-specific grey value-to-stiffness relations as depicted in Figure 14.

Combination of the latter with the porosity maps illustrated in Figure 11, allows for assembly of mechanical property maps as depicted in Figure 15. The latter evidences the very inhomogeneous nature of the organ: The left side of the vertebra organ is less porous, and hence, denser and stiffer

Figure 13. Probability density plots of (a) the macroscopic mass density, (b) the mineral content, and (c) the collagen content.

434 than its right-hand side. Feeding these properties into the differently fine Finite Element models
 435 described in Section 2.6 and Figure 6, yields very similar results in terms of the strain energy
 436 density (see Figure 16), which underlines the sufficient accuracy of the employed Finite Element
 437 meshes. This implies at the same time that the algorithm for using the minimum of three CT-
 438 based porosity values for characterizing one finite element (see Section 2.6) delivers satisfactorily
 439 accurate results. However, there are truly significant differences between the homogeneous and
 440 heterogeneous simulations. Given the employed traction boundary conditions, this indicates that the
 441 homogeneous simulations by far overestimate the stiffness of the investigated organ. Stress levels
 442 obtained, under axial "dead load", in both homogeneous and heterogeneous simulations reach the
 443 level of material yielding (see Figure 18), more pronouncedly so in the heterogeneous simulations
 444 [compare Figure 18(a-c) to Figure 18(d-e)]; while this load level is characterized by a "safety factor"
 445 of about 10 (for homogeneous simulations) and 5 (for heterogeneous simulations) from material
 446 failure [compare Figure 19(a,c,e) to 19(b,d,f)]. Corresponding element-wise increase of the linear

Figure 14. Translation of X-ray attenuation information into tissue elasticity: (a) stiffness tensor components, (b) Young's and shear moduli, and (c) Poisson's ratios; 1,2 ... transverse direction, 3 ... axial direction

elastically determined stress tensors up to the level of material failure is evoking remarkably non-linear strain evolutions, as is seen in Figure 17.

4. DISCUSSION

Combining latest continuum micromechanics models with "universal" composition and mineralization rules for bone, which both were successfully validated against a multitude of physically and statistically independent experimental data, and with X-ray physics and Finite Element modeling, allowed for patient-specific fracture-risk assessment of lumbar vertebrae. In this context, it should be emphasized that we here extended the patient specificity beyond purely geometric aspects, which are somehow defining the current state-of-the-art in computational patient-specific analysis [134–138]; in fact, we here explicitly consider the patient-specific grey value distribution quantifying the spatial X-ray attenuation behavior the scanned organs, and convert the latter into patient-specific microstructural information. The latter then provides, via micromechanics

Figure 15. Property maps in CT slices in the transverse plane:(a-c) axial Young's modulus, and (d-f) axial Poisson's ratio; (g) locations of the mapped cross sections

Figure 16. Convergence study: strain energy density averaged over all finite elements of the investigated FE mesh.

analysis, patient-specific elasticity and strength distributions, which are finally mapped onto the patient-specific geometrical data that we started off with.

Secondly, it is very interesting and instructive to compare the results of our novel method which is solely based on clinical CT data, to results from well established, say "conventional", methods published in the open literature. These methods comprise Archimedes' principle, ultrasound, micro Computed Tomography, macromechanical testing, nanoindentation, and microFE-based tissue elasticity determination.

To begin with, two different techniques, namely Archimedes' principle and evaluation of clinical CT images based on energy-dependent, intravoxel attenuation averaging in conjunction with "universal" compositional rules for soft and hard tissues, delivered the same extracellular bone mass density, when applied to two differently old human vertebrae characterized at two different hospitals. This strongly underlines the organ-specificity, but location- and age-independence of extracellular tissue properties (when averaged over one-to-several-millimeter-sized domains). This remarkable extracellular tissue characteristic is also evident from age-independent nanoindentation moduli on human proximal femora [139–141], as well as from age- and location-independent mineral contents, as obtained from computerized quantitative contact radiography of whole human iliac bone [142], as observed by means of quantitative backscattered electron imaging of iliac and vertebral trabecular samples [143], as seen under Raman microscopy of femoral cortices [144], or as investigated through a Micro Computer Tomograph of an entire human radius [145].

It may also be noteworthy that mass densities obtained *ex vivo*, from samples being stored in a frozen state with temperature and humidity conditions varying quite strongly from those *in vivo*,

Figure 17. Proportional stress loading in chosen finite element [see (a)]; (b) principal stress components and (c) principal strain components as function of the loading degree; (d) stress-strain relation along principal directions

479 agree very well with the mass density data retrieved from *in vivo* scanning protocols. This, however,
 480 agrees very well with evidence put forward by a number of studies [146–149] that the mechanical
 481 properties of bone tissue are preserved through storage, also when bones are frozen. Since the
 482 mechanical properties of bone tissue depend on the tissue's composition and microstructure [30,150]
 483 it appears as very natural that also the composition-related quantities, such as the tissue mass density,
 484 would not be more than negligibly affected by the storage and testing procedure. As a side result, this
 485 allowed us to do without human biopsies when validating our *in vivo* method. In fact, a biopsy from
 486 a young young healthy patient was never considered for ethical reasons, thus ruling out intrusive

Figure 18. Maps of the "yield loading degree" $1/\chi_y$, related to 1 MPa pressure loading; across the sagittal plane through the vertebral body, for (a) homogeneous, and (b) heterogeneous Finite Element model, across the coronal plane, for (c) homogeneous, and (d) heterogeneous FEM; across the transverse plane, for (e) homogeneous, and (f) heterogeneous FEM. (g) 3D representation of the organ and position of the slices. Cross-sectional dimensions are in millimeters

measurement of the patient-specific bone mineral density. This is a normal situation for any analysis performed on clinical data, such as the ones reported by [151].

Figure 19. Maps of the "ultimate loading degree" $1/\chi_{ult}$, related to 1 MPa pressure loading; across the sagittal plane through the vertebral body, for (a) homogeneous, and (b) heterogeneous Finite Element model; across the coronal plane, for (c) homogeneous, and (d) heterogeneous FEM; across the transverse plane, for (e) homogeneous, and (f) heterogeneous FEM. (g) 3D representation of the organ and position of the slices. Cross-sectional dimensions are in millimeters

489 Agreement of our tissue mass density result with all these prior investigations nurtures the
 490 confidence in our new method, which delivers fully patient- and site-specific data on bone tissue
 491 composition, resolved down to the single voxel level. At this stage, it already drives forward the

highly desired knowledge on local bone composition characteristics, as it was achieved by HR-pQCT acquisition schemes on radial bones [64, 152, 153], delivering, as a result, bone mineral densities as averages over cortical or trabecular compartments, with values lying well within the ranges illustrated in Figure 9. Our method, however, while not being dependent on HR-pQCT (which is only applicable to extremities), but just based on standard clinical CTs, gives not only compartment averages, but bone composition gradients resolved down to a voxel size of $0.324 \times 0.324 \times 1.25 \text{ mm}^3$. The identified mass density of 1.67 g/cm^3 underlines that vertebral tissue is remarkably less dense than femoral or tibial tissue, with reported extracellular mass densities lying between 1.9 and 2.0 g/cm^3 [30, 54, 57]. This is consistent with ultrasonic waves traveling slower through vertebral as compared to femoral tissues [51].

Moreover, our mineral contents derived from *in vivo* CT data without the need of any type of phantom, agree remarkably well with *ex vivo* QCT studies as well as ashing studies on vertebral autopsies [154]: for an age slightly below 20 years, the latter reference reports bone mineral contents relating to a similarly aged patient between 70 and 200 mg/cm^3 , a range which well frames the mean value in Figure 12(e). Hence, we provided a new *in vivo* access to values for the so-called "volumetric Bone Mineral Density - vBMD". The latter are very valuable indicators for clinical decisions, as they have been shown to significantly correlate to the occurrence of bone fracture [155], i.e. they are valuable indicators for bone fracture risk. However, such correlations [156–158] do not consider the wealth of knowledge on how materials break, as it has been gained in the fields of material physics and mechanics over centuries.

To the best knowledge of the authors, we here provided the first calibration-free, X-ray physics- and multiscale mechanics-based fracture risk analysis tool applied to human vertebra, based on multiscale elastoplasticity. In this context, it is first interesting to compare the results of the multiscale model as depicted on Figure 5, to corresponding independent experimental results found in the open literature. Namely, the (average) axial normal stiffness component of extracellular bone matrix, amounting to 15.4 GPa , agrees virtually perfectly with the nanoindentation-derived value reported by Wolfram et al. [141], amounting also to 15.4 GPa . At the same time, we observe that the nanoindentation measurements may reveal, when using respective protocols [159], strictly speaking orthotropic material properties, while the micromechanical model employed herein is based on hexagonal symmetry, and hence provides transversely isotropic properties. The question arises how our approximation of orthotropy through transverse isotropy may influence the results of the type of analysis as reported herein. This question can be answered in two independent ways, leading

524 to virtually the same answer. The first answer builds on computing the axial Young's modulus of a
525 piece of orthotropic bone matrix from both the orthotropic stiffness tensor, and from the transversely
526 isotropic approximation of this stiffness tensor, as obtained from rotation around the axial material
527 direction [160]. In the latter reference, it was shown the corresponding difference in axial Youngs
528 moduli computed for the orthotropic data of Ashman et al. [161] amounts to less than 1%; and
529 the same is true for the analogous computations based on the data of [159]. Since only this (axial)
530 Young's modulus enters our Finite Element analyses, we regard the latter's results to be almost
531 entirely independent of the choice of transversely isotropic as compared to orthotropic material
532 properties. The second answer relates to the reliability of formally isotropic analysis as described
533 in Section 2.6: use of such formally isotropic properties as retrieved from the aforementioned
534 nanoindentation campaign indeed delivered Microfinite Element results which agreed particularly
535 well with direct test results of entire vertebrae in both compression and torsion [162]. This further
536 increases the confidence into our method.

537 As regards the plastic load regime, our results for a human lumbar vertebra aged 15 years
538 indicate plastic deformations to even occur under normal physiological loading, agree well with
539 the growing evidence of low-level plasticity in bone tissue, demonstrated by means of mechanical
540 experiments and Finite Element simulations of trabecular bone [163, 164]. The safety factor against
541 ultimate fracture is found to be around five, corresponding to an ultimate load amounting to 4614 N,
542 which lies well within the broad range of values determined experimentally on different human
543 vertebrae [1–4]. Our ultimate load particularly well agrees with the values of ultimate load measured
544 on vertebrae of similar mean BMD [1, 3, 11], and for a bone of similar mean porosity [2, 10]. It
545 is very interesting that our failure also agrees very well with those determined for femurs, both
546 experimentally [16] and by means of FE strength computations [17]. This indicates larger domains
547 of the skeleton to be designed for bearing the same level of loading. Thereby, the critical load
548 may well be attained in extreme sport activities, as compressive forces of 8676 N in the block
549 action of an American football player, 7500 N during a golf swing, or 7756 N during torso weight
550 lifting [165]. The question may arise whether the large regions close to yielding under kind of a
551 "light loading" makes sense from an evolutionary biology standpoint. The answer may actually
552 be affirmative, given the evolution of the spine as a lightweight shock absorbing organ system. In
553 fact, dissipative mechanical behavior of vertebral bone even under light loads seems very much
554 consistent with spine's function as kind of a "shock absorber". Accordingly, even at low loading,
555 the mechanical work put into the system would not be entirely recoverable upon unloading, but

be partially dissipated into heat. Identification of related yielding phenomena critically relies on the advanced multiscale formulation used herein, in order to describe the elastoplastic behavior of bone, in particular at the scale of the extracellular bone matrix. The plastic behavior of bone at low load levels at this small scale has been very recently shown also by a direct experimental method where single micron-sized samples glued onto a rigid substrate have been loaded and unloaded in the compressive regime [166]. A particular feature of our new method is the consistent consideration of heterogeneous elasticity and strength properties throughout the organ - it is remarkable that our bottom-up approach value for the Young's modulus of the extravascular space [see Figure 14(a) and Eqn.(27)] compares well to data stemming from top-down back-computations reported in the literature [167]; and the importance of heterogeneity for fracture loads has been repeatedly stated [168]. In our case, the heterogeneity results from the bone remodeling induced by the natural asymmetry of the human body, with the right part of the middle section of the vertebra being less porous, and therefore stiffer than the left-hand part, see Figure 6(a). Resulting stress concentrations govern the organ's compliance and safety margin against fracture: stiffness and strength properties based on an averaged homogeneous vascular porosity would overestimate the strain energy density by a factor of 5, and the safety factor by a factor of 2. This key role of tissue heterogeneity in the mechanics of the investigated biological system is fully consistent with similar earlier findings on a variety of other tissues, such as human and murine femoral bone [43, 169, 170], primate skull [171], human patellar cartilage [172], or human arterial wall tissue [173].

Our method critically depended on a successful micromechanical representations of bone microstructure for mechanical property predictions, which does not resolve the material up to the highest resolution ever possible, but rather focus on reliable consideration of the mechanically most important micromorphological features. While in the present case, the micromechanics model was set within the framework of continuum micromechanics or random homogenization theory, the same philosophy can also be followed within alternative theoretical frames, such as that of lattice models [112]. At the same time, our method impressively confirms the increasing expectations of the clinical environments with regards to advanced numerical methods, especially those based on the Finite Element method [174].

It is also appropriate to discuss potential limitations of the new method presented here. The phantom-free CT data evaluation critically relies on the existence of two distinguishable peaks in the histogram of grey values as depicted in Figure 7, these peaks being related to the adipose and the soft tissue, respectively. Certain pathological conditions or poor resolution images may

render these two peaks as non-distinguishable. In this case, the scanning of the clinically interesting part of the body, together with a piece of material with known chemical composition (such as a phantom used for standard CT data calibration purposes) may deliver access to a second grey value with known corresponding attenuation coefficient, so that a system of equations similar to that of Eq. (2) and (3) may again provide access to the energy-dependent functions $a(\mathcal{E})$ and $b(\mathcal{E})$. Such a "phantom-based" X-ray energy determination process has been described in greater detail for ceramic biomaterials [44]. In its current formulation, the presented method is realized for the case if the cortical shell is thinner than the voxel size, see procedure of Section 2.2. This procedure would need modification if thick cortical shells, i.e. such with thicknesses spanning one entire or even several voxels edge lengths, would appear in the scanned domain. Actually, the maximum grey value would then coincide with the extravascular grey value GV_{ev} , so that Eq. (8) to (12) would become superfluous, while the rest of the analysis would stay unaltered.

The practical impact of our study is deemed to lie in its ability to allow for an enhanced exploitation of standard clinical CT data with low radiation exposure to the patient; thereby revealing information which would otherwise stay unnoticed. Without any additional efforts with respect to standard medical examinations, the method described here allows for unprecedented information on the fracture risk of the scanned vertebrae. Such information may be deemed essential in the process of seeking second opinions in diagnosis or treatment processes [175].

As a side effect of the clinical orientation of our protocol (we are dealing with data from a living patient), the *in vivo* scanned object could not be used for further *in situ* testing. Still, the identified mass density agreed very well with that obtained from a cadaveric specimen. This agreement further confirms earlier statements on the temporal and spatial invariance of species- and organ-specific adult bone tissue mass density when averaged over millimeter-sized domains [37, 139–145].

Finally, one might discuss on how to proceed this research work so as to eventually integrate it into a real-life clinical setting. One administrative problem in this context could be the integration of commercial Finite Element software as employed here, together with several in-house codes produced at the university, in current workflow systems employed in the clinical everyday life; together with the software and hardware computer power needed for running advanced medium-scale FE computations as reported herein. In this context, advanced computational techniques such as automated object-based image analysis [176]; or statistical shape models for active feature selection [177], are gaining popularity. A more conceptually fundamental way to overcome the aforementioned challenge could be to substitute the full 3D FE analyses by similarly precise,

yet computationally and commercially less expensive advanced beam model solutions. In fact, preliminary work in our research group (which is obviously beyond the scope of the present paper) indicates that advanced "sandwich beam"-type theories turn out as theoretically challenging, but highly effective alternatives to Finite Element models. They are particularly effective when it comes to tackling load cases which go beyond the basic situation of a constant pressure acting on the upper boundary of the investigated vertebra as described in this paper. This can be already sketched here, in order to give a first-order approximation of how the safety factors would roughly change when a typical bending moment of some 40 Nm [178], in addition to the constant pressure of 1 MPa as discussed earlier, would be applied to the studied vertebra. Approximating the vertebral cross section as shown in Figure 12 as a homogeneous ellipse with some 50 mm width and 33 mm height results in a minimal area moment of inertia of $I=33^3 \times 50 \times \frac{\pi}{4} = 1.4 \times 10^6 \text{ mm}^4$, so that the normal stresses due to bending can be roughly estimated as $\sigma=40/(1.4 \times 10^{-6}) \times 16.5 \times 10^{-3} \approx 500 \times 10^3 \text{ Pa}=0.5 \text{ MPa}$. Hence, a typical bending mode on top of the compressive loading studied throughout this paper leads to an increase of the pseudo-uniaxial stress state of Figure 17 by around 30%; i.e. the overall safety would be expected to be around four in combined axial-bending load.

5. CONCLUSION

Based on consistent integration of micromechanics, Finite Element analysis, and X-ray physics, conventional clinical CT data alone allow for reliable determination of tissue mass density and mineralization degree, as well as engineering mechanics-based fracture risk analysis considering both patient-specific loading and microstructurally governed conditions of mechanical integrity.

CONFLICT OF INTEREST STATEMENT

The authors Romane Blanchard, Claire Morin, Andrea Malandrino, Alain Vella, Zdenka Sant, and Christian Hellmich hereby declare that they have no conflict of interest.

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NOMENCLATURE

Variables

a	... slope of linear relation between grey values and X-ray attenuation coefficients
A	... area
$\mathbb{A}$	... strain concentration tensor
b	... intercept of linear relation between grey values and X-ray attenuation coefficients
$\mathbb{C}$	... stiffness tensor
C_{ijkl}	... stiffness tensor component
C_{ijkl}^{-1}	... compliance tensor component
$\mathbb{D}_{ic,\Phi\Theta}$	... influence tensor accounting for the influence of plastic strains in the (Φ, Θ) -oriented mineral phase on the total strains in the inter-crystalline pores
$\mathbb{D}_{\phi\theta\Phi\Theta}$	... influence tensor accounting for the influence of plastic strains in the (Φ, Θ) -oriented mineral phase on the total strains in the (ϕ, θ) -oriented phase
E	... Young's modulus
$\mathcal{E}$	... photon energy
$\mathbf{E}$	... macroscopic strain
f_i^j	... volume fraction of phase i (if specified, in the RVE j ; otherwise, in the macroscopic RVE)
$\mathcal{F}$	... yield function
$\mathcal{G}$	... flow potential
G_i	... shear modulus of constituent i
GV	... X-ray attenuation-related grey value
l	... characteristic length
$\mathbb{I}$	... fourth-order identity tensor
k_i	... bulk modulus of constituent i
l	... characteristic length
N	... total number of finite elements
N_C	... number of constituents in the volume (voxel)
$\mathbb{P}$	... Hill tensor or morphological tensor
R	... ratio
V_n	... volume of the n -th finite element
W	... weight

- $\mathbf{x}$... (microscopic) position vector inside the RVE
- β ... ration between compressive and tensile yield stress of HA
- δ_{ij} ... Kronecker delta
- $\boldsymbol{\varepsilon}$... microscopic strain tensor
- $\dot{\lambda}$... plastic multiplier
- μ_i ... X-ray intensity attenuation coefficient of constituent i
- ν ... Poisson's ratio
- $\boldsymbol{\xi}$... displacement
- ρ_i ... "real" mass density of material constituent i
- $\rho_i^{*,j}$... apparent mass density of material constituent i per volume of space j
- $\bar{\rho}$... average mass density
- $\boldsymbol{\sigma}$... microscopic stress
- $\boldsymbol{\Sigma}$... macroscopic stress
- ϕ_i ... porosity of constituent i
- ϕ_i^j ... porosity of space i (if specified, in the RVE j ; otherwise, in the macroscopic RVE)
- χ ... risk factor
- ψ ... macroscopic strain energy density
- $\bar{\psi}$... strain energy density averaged over organ

Subscripts

- 1 ... in radial direction
- 2 ... in circumferential direction
- 3 ... in axial direction
- a ... dried in air
- $bone$... of bone
- col ... of collagen
- $cort$... of cortical shell
- cyl ... of cylindrical inclusions
- dev ... deviatoric part
- ec ... of extracellular bone matrix
- ef ... of the extrafibrillar space
- end ... of the bony endplate

ev ... at extravascular scale

fat ... of adipose tissues

HA ... of hydroxyapatite

H₂O ... of water

H₂O + ncp ... of water and non collageneous proteins

hom ... homogenized

ic ... of the inter-crystalline space

lac ... of lacunae

macro ... of macroscopic bone

max ... maximum value

max - 1 ... penultimate maximum value

org ... of organic matter

organ ... of the full organ

s ... submerged in water

soft ... of soft tissues

sph ... of spherical inclusions

thr ... at threshold value

vas ... of vascular porosity

vol ... volumetric part

voxel ... of one voxel of the CT image

ult ... ultimate

y ... at the start of yielding

$\phi, \theta, \Phi, \Theta$... Euler angles defining the orientation of the crystal needles (cylindrical mineral phases)

Superscripts

I, II, III ... eigenvalues

ec ... in the extracellular bone RVE

ef ... in the extrafibrillar space

EV ... statistically expected value

iso ... in an isotropic phase

p ... plastic

trab ... of trabecular bone

ult ... ultimate

up ... upscaled

y ... at the start of yielding

∞ ... at infinite boundary of auxiliary matrix in Eshelby's inclusion problem

Mathematical symbols

∇_s ... symmetrized nabla operator

$:$... second-order tensor contraction

fig1

fig3

fig4

fig5

fig6

fig7

fig9

fig10

fig11_rev

fig12

fig14

fig15_rev

fig16

fig17

fig18

fig19