

Semi-Automated μ CT-Based Modeling of Vertebral Bony Endplate Microporosity and
Mechanical Properties: Linking Microstructure to Inferred Mechanical Performance

Ahmad Alminnawi^{a,b,c*}, Gabriele Nasello^{a,b}, Katherine B. Crump^{d,e,f}, Benjamin Gantenbein^{d,e,f},
Jerome Noailly^g, Liesbet Geris^{a,b,c*}

^aPrometheus, Division of Skeletal Tissue Engineering, KU Leuven, Herestraat 49, 3001 Leuven,
Belgium. Tel. +32(0)16373223, liesbet.geris@kuleuven.be, ahmad.alminnawi@kuleuven.be

^bBiomechanics Section, KU Leuven, Celestijnenlaan 300, 3001 Leuven, Belgium.
liesbet.geris@kuleuven.be, ahmad.alminnawi@kuleuven.be

^cGIGA Molecular and computational biology, University of Liège, Av. Hippocrate 1/11, 4000
Liège, Belgium. Tel: +32(0)495639951, liesbet.geris@uliege.be, ahmad.alminnawi@uliege.be

^dTissue Engineering for Orthopaedics & Mechanobiology, Bone & Joint Program, Department for
BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, Switzerland

^eDepartment of Orthopaedic Surgery and Traumatology, Inselspital, Bern University Hospital,
Medical Faculty, University of Bern, Bern, Switzerland

^fGraduate School for Cellular and Biomedical Sciences (GCB), University of Bern, Bern,
Switzerland

^gBCN MedTech, Department of Engineering, Universitat Pompeu Fabra, 08002 Barcelona, Spain

* Corresponding author: Ahmad Alminnawi, ahmad.alminnawi@kuleuven.be

1. Abstract

The vertebral bony endplate (BEP) plays a role in regulating both mechanical load transfer and nutrient transport to the intervertebral disc, functions strongly influenced by its microstructural porosity. Variations in porosity impact bone strength, elasticity, and solute diffusion, affecting its overall mechanical competence.

A semi-automated modeling workflow was established to quantify pore geometry and connectivity of the BEP and relate them to zone-specific mechanical and transport properties using relationships previously established in the literature. Six to eight BEPs from four bovine subjects were imaged using micro-computed tomography. The central zone and two peripheral zones of each BEP were segmented and their microstructure, including tortuosity, porosity, and pore radii, were quantified using the pipeline to assess BEP heterogeneity. These metrics were then used to infer the BEP's zone-dependent density, Young's modulus, compressive strength, and shear modulus.

The BEPs showed spatial, intra-subject, and inter-subject morphological variability, which affected their predicted mechanical and transport properties. These findings highlight the limitations of purely idealized BEP representations *in silico* simulations and support the incorporation of uncertainty modeling strategies that account for physiologically relevant variations in the BEP. This semi-automated modeling approach represents a tangible step toward more realistic *in silico* simulations of vertebral endplate function.

Keywords: Vertebral bony endplate, Microstructure, 3D Modeling, Pore diameter, Permeability, CT scan, Intervertebral disc

2. Introduction:

Degeneration of the intervertebral disc (IVD) and vertebral endplate is associated with compromised mechanical load transmission and impaired nutrient transport, contributing to disc degeneration, low back pain, and adjacent tissue failure¹⁻⁴. The vertebral endplate is divided into a bony endplate (BEP), which lies on the subchondral trabecular bone (STB) of the vertebral body (VB), followed by a layer of cartilage called the cartilaginous endplate (CEP) that is usually calcified towards the BEP and uncalcified towards the IVD. The entire endplate plays a central role in the aforementioned pathological processes by governing both load transfer and solute exchange to the avascular IVD. These functions are highly sensitive to the endplate's microstructural porosity and connectivity, which has been investigated experimentally⁵⁻⁸ as well as through computational modeling and simulations⁹⁻¹². Even modest alterations in endplate porosity have been shown to substantially reduce mechanical strength while simultaneously modifying transport pathways^{10,13-15}, highlighting the need for accurate microarchitectural characterization.

The BEP, despite its thinness (0.2 - 1 mm¹⁶⁻¹⁸) (Figure 1), contributes 30-60% of bone mass in the VB and bears 45-75% of the compressive loads¹⁹. In dense bone, even modest increases in porosity can disproportionately reduce tensile and compressive strength, elasticity, and toughness¹⁹⁻²³. For example, porosity accounts for approximately 70% of the reduction in elastic modulus and more than half of the decrease in yield stress of cortical bone²¹. It is well established that increasing porosity leads to a reduction in bone strength and elasticity, typically following power-law relationships^{21,24-27} as these properties are affected by the structure, geometry, and organization of the pores²⁷⁻²⁹.

Beyond its load-bearing role, the BEP has a highly porous microarchitecture, which is specialized for fluid transport and load distribution^{14,15,30-33} as it is the last vascular structure before the IVD. The BEP-CEP interface is considered the primary pathway for nutrient delivery and waste removal in the IVD, the largest avascular structure in humans^{34-38 15,35,39}. Porosity is increasingly regarded as a quantifiable and predictive marker of bone fragility, with potential to identify at-risk individuals not captured by standard osteoporosis classifications^{40,41}, especially since degeneration, microcracks, and Schmorl's nodes are common through the BEP and are linked to disc pathology⁴²⁻⁴⁶. As BEP microstructure directly governs mass transport between the culture medium and the intervertebral disc, interpreting experimental measurements after organ culture perturbation

without the microstructural information at the BEP level is inherently limited as was shown by Crump et al.⁷.

Recent advances in imaging now enable accurate visualization of BEP morphological heterogeneity. Micro-computed tomography (μ CT) has become the gold standard for high-resolution bone microarchitecture visualization at the sub-micron scale, and CT-based analysis of BEP pore geometry and connectivity can support model-based predictions of its mechanical and transport parameters. A computational modeling approach offers key advantages over direct experimental measurement. It is non-destructive, preserving sample integrity for downstream analyses such as histology and immunohistochemistry. More importantly, it enables flexible spatial segmentation within each sample, allowing spatially resolved analysis and yielding a richer, more comprehensive characterization of spatial, intra-subject, and inter-subject variations in mechanical and transport properties. This facilitates detailed assessment of tissue heterogeneity and supports the integration of organ culture models with computational simulations. In this study, we applied a semi-automated pipeline to analyze 84 zones obtained from 28 BEPs across four bovine subjects. The workflow enabled reproducible segmentation of bone microporosity and direct quantification of morphological parameters, including tortuosity, porosity, and pore radii. These measures were then used to infer pore-dependent mechanical and transport properties, including permeability, tortuosity, Young's modulus, compressive strength, shear modulus, and density across the BEP samples. These properties, coupled with the generated 3D meshes, provide a foundation for future sample-specific computational models and enable mechanistic simulation-based predictions of BEP influences on the disc health, for example, within IVD organ culture models⁷.

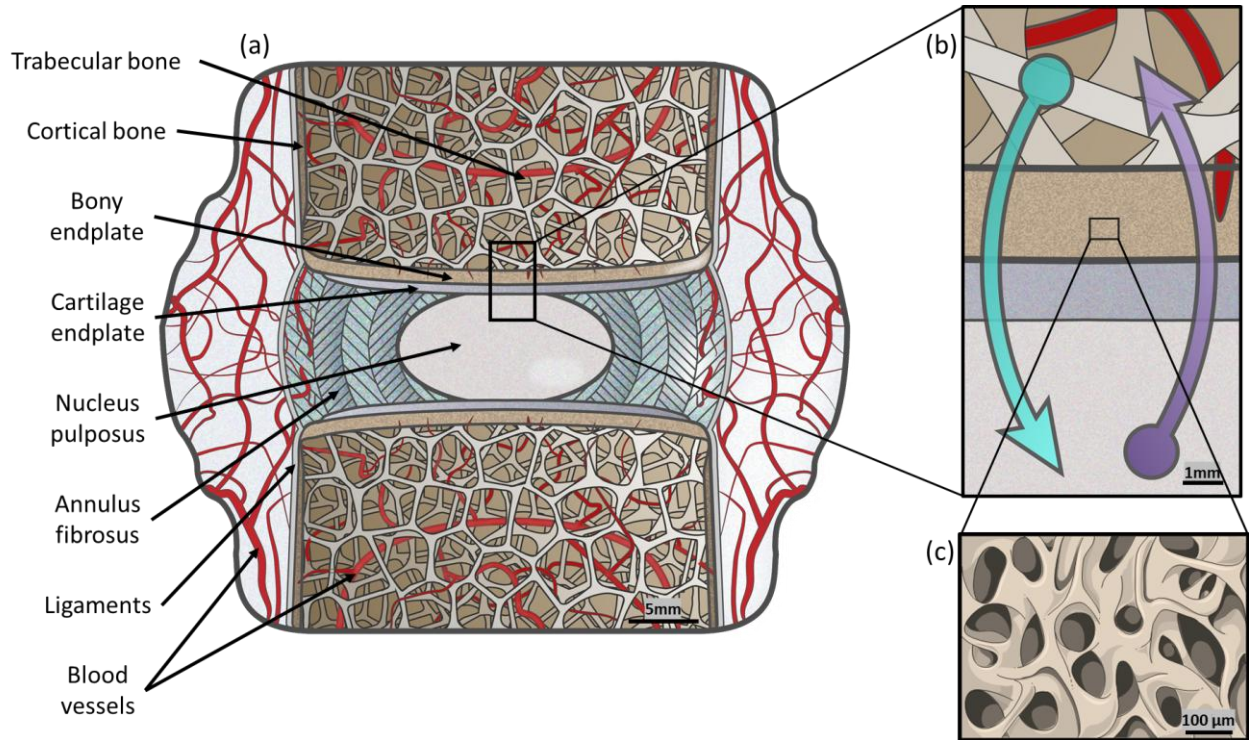


Figure 1: Intervertebral disc sandwiched between its vertebral bodies (a) showing the path of nutrients (blue) and waste (purple) between the disc and the blood vessels in the vertebral bodies (b), diffusing through the microporosity of the bony endplate (c)

3. Methods

3.1. Biological BEP acquisition and microstructural imaging

3.1.1. Bovine bony endplate harvesting and preparation

In this study, bovine BEPs were used to test and validate the pipeline, as they have been extensively used as a substitute for human studies in lumbar disc research because of their availability and experimental reproducibility^{47–51}. To minimize ethical and animal-welfare concerns, four fresh, full-length bovine tails were obtained from the local slaughterhouse on the day of slaughter, and VBs were harvested from each tail using modified protocols previously published^{52,53}. Briefly, the tails were acquired without skin to reduce the risk of contamination and sterilized with betadine solution before being placed under the laminar flow hood for processing. All muscles, ligaments, and outer connective tissues were removed using a scalpel and tweezers. Intact VBs, including BEP, STB, and cortical bones, were extracted from the middle section of each tail. These VBs were then placed into containers with PBS (Phosphate-Buffered Saline) solution and shaken 3-6 times

until the bone appeared white, indicating the complete removal of the bone marrow and blood clots. On average, two 8 mm punches were then taken from each VB (Figure 2), at the caudal and cranial BEP sites, using a T-handle bone trephine biopsy punch with ejector. After accounting for samples lost during handling, a total of 28 VB punches were harvested (6-8 per subject). As different tail segments were obtained from the slaughterhouse for each bovine subject, vertebral level could not be standardized across samples and the punch numbers were therefore assigned as identifiers regardless of vertebral level. Using μ CT imaging and 3D meshing, three microstructural parameters were quantified, with porosity as the primary focus due to its dominant role in governing transport and mechanical properties^{10,54}, alongside tortuosity and mean pore radius.

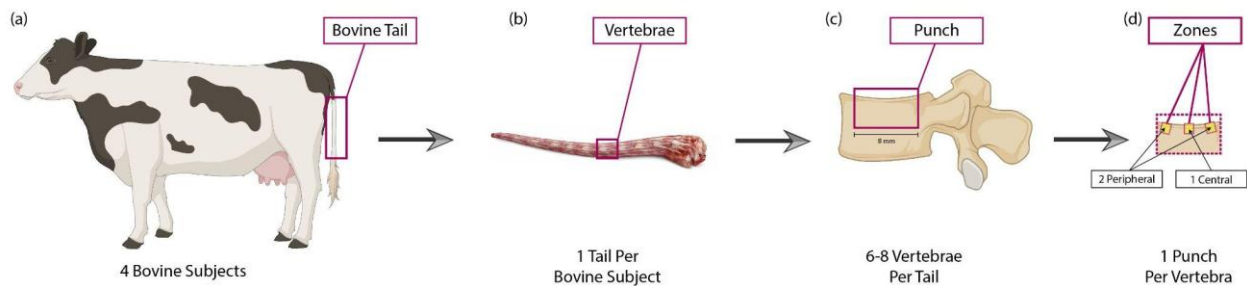


Figure 2: Workflow illustrating the sample preparation: a bovine tail was harvested (a) and processed by removing skin, muscles, and ligaments (b), followed by isolation of vertebrae (c). Cylindrical punches were extracted to be scanned using micro-CT, and three zones were highlighted (one central and two peripheral) for subsequent analysis. The bovine and vertebra illustrations were created in BioRender. Ivanets, M. (2026) <https://BioRender.com/p4sbsp46>.

3.1.2. μ CT imaging

The VB punches were fixed in 4% formaldehyde for 48 hours and subsequently immersed in a 40% ioxaglate (Hexabrix[®]) solution for up to two weeks. Hexabrix, an anionic, low-osmolarity, and low-viscosity contrast agent⁵⁵, is electrostatically repelled by the negatively charged glycosaminoglycans (GAGs) in cartilage^{56,57}. This differential distribution enhances contrast between calcified and non-calcified regions⁵⁸, thereby improving tissue delineation in the scans. Each VB punch was subsequently wrapped in parafilm and scanned using a high-resolution μ CT scanner, the Phoenix Nanotom[®] by Baker Hughes (Waygate Technologies). The punches were scanned with in-house optimized settings using a diamond-coated tungsten target and a 0.2 mm Aluminum filter at a voxel size of 4-7 μ m. Each VB punch was scanned at a voltage of 60 kV and a current of 170 μ A at an exposure time of 500 msec. 1800 images were acquired over 360° using

the fast scan mode. During reconstruction, the beam hardening correction (bhc) was set to 8 and the inline median filter, the ROI CT filter, and the filter volume were turned on. 16-bit TIFF files were exported immediately after reconstruction.

Bone porosity consists of two distinct systems: vascular channels (Haversian and Volkmann canals) and the lacuno-canalicular network. However, connected vascular porosity is the dominant contributor to mechanical and transport properties, as the volume fraction of lacunae and canaliculi is negligible in comparison⁵⁹. Therefore, the pore network of interest in this study primarily comprises the larger vascular channels, which are resolvable at a voxel size of 4-7 μm .

3.2. Semi-automated pipeline

The full set of algorithms covering all tools mentioned below is available for download on our biomech website at <http://www.biomech.ulg.ac.be/software/> and in the public GitHub repository⁶⁰, along with a user manual and the full unprocessed output dataset to support reproducibility. The Python-based pipeline (Figure 3a), implemented in Jupyter notebooks and integrated with 3D Slicer 5.0.3^{61,62}, generates 3D pore structures from μCT datasets, through a nine-step procedure. The steps are numbered 00 to 08 in the user manual and the scripts are numbered accordingly in the repository. A description of the steps is provided in the following subsections.

3.2.1. Structure extraction and mesh generation pipeline

In preprocessing, the exported TIFF files were imported to 3D Slicer and stacked using the ImageStacks module of SlicerMorph⁶³. They were converted into two volumetric datasets (.nrrd files), one low quality dataset with voxel spacing of 16-28 μm for quick manual handling and confirmation (script 00), and another high-quality dataset with voxel spacing automatically extracted from metadata to be used in the pipeline (script 01). Segmentation was performed using a two-step automatic thresholding strategy, optimized by evaluating 12 global thresholding algorithms, that were Otsu⁶⁴, Huang⁶⁵, Intermodes⁶⁶, IsoData⁶⁷, KittlerIllingworth⁶⁸, Li^{69,70}, MaxEntropy⁷¹, Moments⁷², RenyiEntropy⁷¹, Shanbhag⁷³, Triangle⁷⁴, and Yen⁷⁵ (Figure 3b). Threshold ranges were determined on a sample-specific basis. For each sample, all the automatic thresholding methods were evaluated (Script 02), each producing a candidate intensity threshold. For the lower bound, the candidate threshold closest to the first local minimum of the intensity

histogram was selected. Voxels with intensities below this threshold were subsequently excluded. The thresholding algorithms were reapplied to the truncated intensity distribution (Script 03). For the upper bound, the candidate threshold closest to the second peak of the histogram was selected, provided that the associated peak frequency was lower than that of the lower-bound region. Two intensity intervals were defined: first, from minimum intensity to the lower bone threshold as “void pores”, and second, from the upper bone threshold to maximum intensity as “Hexabrix-filled pores”. The voxels that fall within these two ranges were segmented and merged to generate a unified pore segmentation. Since the void and Hexabrix segmentation intensity ranges are separated by bone intensities, and to mitigate partial volume effects at pore interfaces, a one-voxel morphological dilation was applied to the unified segmentation.

To reduce the computational load associated with analyzing the vertebral BEP samples, and to evaluate the heterogeneity of each sample, cylindrical regions of interest (ROIs) were defined interactively within each scan to isolate three zones of the BEP: one central and two peripheral zones (Figure 3c). This step was done semi-manually on the low-quality dataset by drawing a line that represents the starting point of the ROI and the direction of the cylinder base (script 04). Each ROI has a height of 1 mm and a radius of 0.5 mm and is labeled as ‘zone’ in the following sections. The unified pore segmentation, corresponding ROIs, and the high-quality dataset were subsequently used (script 05) to reconstruct the local pore geometries into high-resolution STL surface meshes for the 84 zones.

To ensure high quality, the meshes were processed using MeshLab (PyMeshLab 2023.12.post2t library) (script 06). Processing included surface reconstruction⁷⁶ using a screened Poisson approach (depth = 8, scale = 1.1), and standard operations which included removal of duplicate and zero-area elements, merging of close vertices, elimination of non-manifold configurations (e.g., T-vertices and non-manifold edges) and self-intersections, as well as reclosing the resulting holes (if present) to ensure surface continuity. Small disconnected components were discarded using size-based filtering (20% of the zone bounding box) and, finally, meshes were checked for watertightness (Figure 3d).

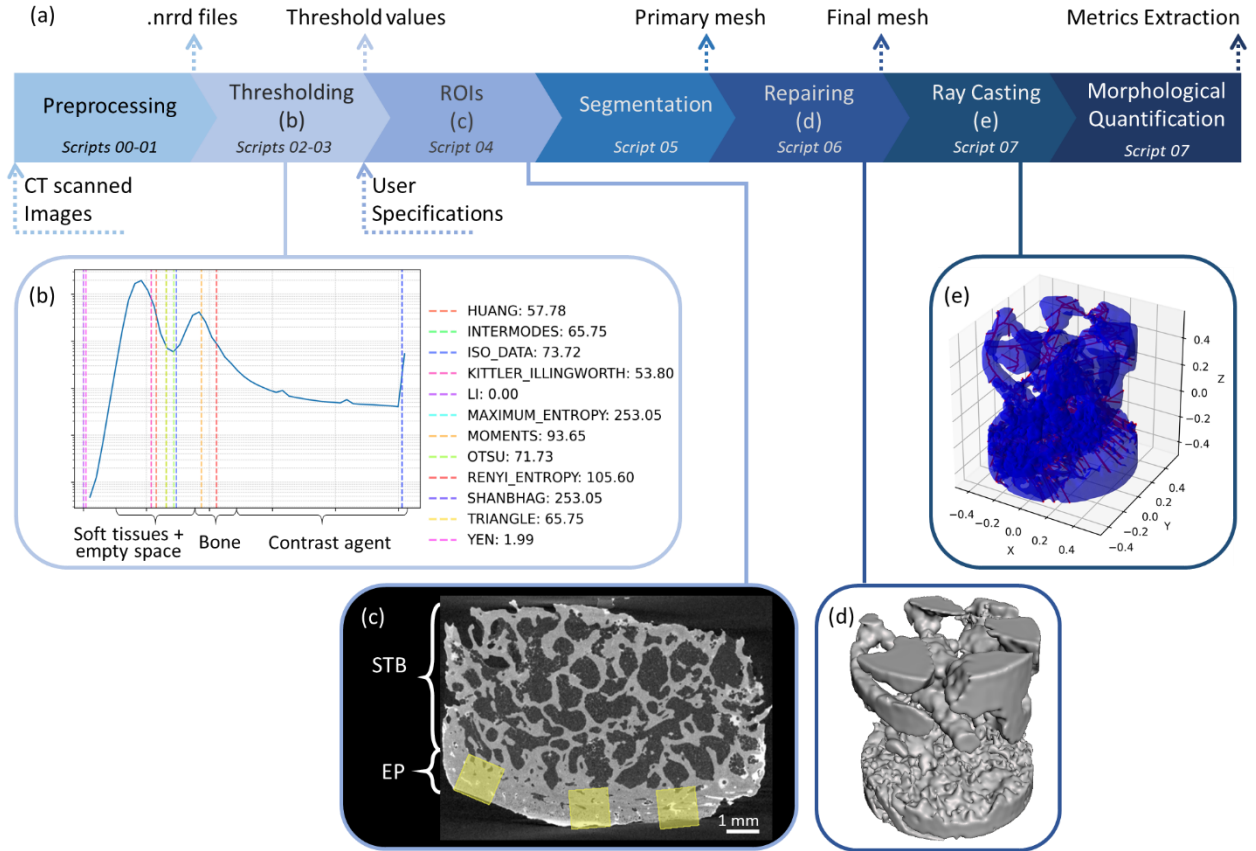


Figure 3: Porous tissue structure extraction and mesh generation workflow. (a) Overview of inputs and outputs at each step. (b) Automatic thresholding histogram produced by all twelve methods, showing the three ranges: empty space combined with soft tissues, bone, and contrast agent. (c) a VB punch with the 3 ROI zones, the central and two peripheral ones. (d) The cleaned final mesh generated for a zone in the VB punch, representing the pores. (e) The skeletonization and ray casting approach measuring pore radii.

3.2.2. Geometric and morphological quantification

Following mesh reconstruction, geometric and morphological parameters were computed directly from the mesh to ensure consistency with any downstream computer simulations (not included in this study). Porosity was calculated as the ratio of pore mesh volume to the total volume of the cylindrical ROI. A wavefront-based approach⁷⁷ was used to skeletonize the mesh and produce the centerlines that represent the pore network (Figure 3e). Local pore radii were estimated by casting perpendicular rays from each skeleton anchor to the mesh surface. A ray-triangle intersection test was performed, and the apparent pore radius was computed as the distance between the anchor and the first intersection with the mesh surface. Porosity and pore size alone are insufficient to characterize fluid and particle transport in porous media. A critical prerequisite is percolation,

defined as the presence of at least one continuous pore pathway from the caudal to the cranial surface and enabling through-flow categorized as “percolating pores”. Accordingly, vertices at the cranial-most and caudal-most extents (Figure 3e, top and bottom, respectively) were identified using a 2% tolerance (0.02 mm). Pore path lengths were then determined by computing all unique paths within the skeletonized graph that connected these two vertex groups using a depth-first search initiated from branch endpoints (Script 07). Absence of percolation suggests that the sample was not permeable at that zone.

Scripts 00 to 07 can be executed independently for each sample or executed using Papermill (Scripts A to D), which enables parameterization and batch execution of the pipeline across multiple samples as explained in the user manual.

3.2.3. Porosity-dependent mechanical and transport properties estimation

In geology and material sciences, tortuosity characterizes the path of fluid flow through porous media with complex microstructures, thereby influencing mass transport⁷⁸⁻⁸¹. Geometric tortuosity quantifies the deviation of pore pathways from a straight line, reflecting the extent to which they twist, turn, or branch, thereby increasing the effective path length relative to the zone thickness. It is commonly quantified as the ratio of the actual path length a molecule or fluid must traverse within the porous network to the straight-line thickness of the material (Equation 1). By definition, this ratio is always greater than or equal to 1, with a value of 1 representing an ideal straight path and non-percolating zones would have an undefined (infinite) tortuosity. In our case, the actual path length was defined as the average percolating pore centerline length, while the straight-line path was taken as the zone height to calculate tortuosity τ :

$$\tau = \frac{L}{H} \quad , \quad (1)$$

with L being average pore centerline length, and H being the zone height.

The Kozeny-Carman equation is widely used to predict permeability in porous media and has been proven to be accurate for the BEP of the IVD¹⁰. Although it exists in several forms, all are derived from principles of classical Navier-Stokes fluid mechanics. When tortuosity is explicitly considered, the equation can be expressed as follows:

$$k = \frac{\varphi^3}{c(1-\varphi)^2 S^2 \tau^2} \quad , \quad (2)$$

where k is the intrinsic permeability, φ is the porosity, and c is the Kozeny-Carman dimensionless constant. S is the specific surface area of the pores defined as the ratio of the surface area of the solid-pore interface (A_i) to the volume of the solid phase (V_s).

$$S = \frac{A_i}{V_s} \quad (3)$$

The volume of the solid phase can be calculated as the difference between the ROI volume and the volume of the pores.

The Kozeny-Carman dimensionless constant reflects the influence of pore shape and arrangement on flow resistance. It is commonly assigned a value of 5 in studies of bone tissue engineering⁸²⁻⁸⁴. The intrinsic permeability is expressed in units of area (m^2 in SI) but is more widely reported in Darcy (D), where $1 \text{ D} \approx 0.987 \times 10^{-12} \text{ m}^2$ ^{85,86}. One Darcy corresponds to the permeability of a 1 cm^2 cross-section, 1 cm in length, that permits a flow of 1 cm^3/s of a fluid with a viscosity of 1 cP under a pressure gradient of 1 atm/cm.

In this study, mechanical and transport properties were predicted from morphology using established empirical models. Porosity significantly influences all properties of the bone. Density (ρ) decreases linearly with increasing porosity, while elasticity (Young's modulus E), strength (compressive strength σ_u), and shear behavior (Shear modulus G) exhibit exponential decay, consistent with the Spriggs equation²⁴⁻²⁷. In addition, the three moduli depend on both density and strain rate ($\dot{\epsilon}$) of the measurement protocol, as exemplified in the equations below (Table 1).

$$\frac{\rho}{\rho_0} = (1 - \varphi) \quad (4)$$

$$E = A_E \cdot \rho^{a_E} \cdot \dot{\epsilon}^{b_E} + B_E \quad (5)$$

$$\sigma_u = A_\sigma \cdot \rho^{a_\sigma} \cdot \dot{\epsilon}^{b_\sigma} + B_\sigma \quad (6)$$

$$G = A_G \cdot \rho^{a_G} \cdot \dot{\epsilon}^{b_G} + B_G \quad (7)$$

In these equations, A , a , b , and B are empirical constants characterizing the relationship of each modulus with density and strain rate. ρ_0 ($=2.2 \text{ g/cm}^3$) denotes the density of bone without porosity

derived from cortical bone, which typically has a porosity around 5% and is approximately an order of magnitude lower than that of the BEP^{54,87–89}.

The BEP is described as a layer of densely fused trabeculae^{13,16,90,91} and commonly assigned homogeneous and isotropic material properties as is the case for trabecular bone in the literature¹³. Accordingly, trabecular bone empirical relationships were applied at the mesoscale on the BEP zones. The relationship between porosity and density is consistent across multiple studies in the literature. An explicit equation for shear modulus (G) has been reported in only one study and was adopted here⁹². For Young's modulus (E) and compressive strength (σ_u), multiple studies propose differing parameters (summarized in Table 1); therefore, 3D regression models were created using nonlinear curve fitting to arrive at consensus relationships to predict the corresponding moduli.

272 Table 1: Reported mathematical relationships between geometric properties and trabecular bone mechanical properties. E:
273 Young's modulus; ρ : Density; σ_u : Compressive strength; $\dot{\epsilon}$: Strain rate

Mechanical property	Reference	equation type	Model	Correlation coefficient	sample origin
Elasticity, E (MPa)	Carter DR. ⁹³	Power Function ($E = a \cdot \rho^b \cdot \dot{\epsilon}^c$)	$E = 3790 \cdot \rho^3 \cdot \dot{\epsilon}^{0.06}$	-	human tibia
	Linde F. ⁹⁴ _1	Power Function ($E = a \cdot \rho^b \cdot \dot{\epsilon}^c$)	$E = 2232 \cdot \rho^{1.56} \cdot \dot{\epsilon}^{0.047}$	0.84	human tibia
	Linde F. ⁹⁴ _2	Near Density ($E = (a + b \cdot \rho) \cdot \dot{\epsilon}^c$)	$E = (-321 + 2164 \cdot \rho) \cdot \dot{\epsilon}^{0.047}$	0.84	human tibia
	Linde F. ⁹⁴ _3	Quadratic Density ($E = a \cdot \rho^2 \cdot \dot{\epsilon}^c$)	$E = 3101 \cdot \rho^2 \cdot \dot{\epsilon}^{0.044}$	0.82	human tibia
	Linde F. ⁹⁴ _4	Cubic Density ($E = a \cdot \rho^3 \cdot \dot{\epsilon}^c$)	$E = 6093 \cdot \rho^3 \cdot \dot{\epsilon}^{0.034}$	0.66	human tibia
	Linde F. ⁹⁴ _5	Linear Strain Rate ($E = \rho^b \cdot (a + c \cdot \dot{\epsilon})$)	$E = \rho^{1.43} \cdot (1534 + 103 \cdot \dot{\epsilon})$	0.89	human tibia
	Linde F. ⁹⁴ _6	Carter and Hayes Type ($E = a \cdot \rho^3 \cdot \dot{\epsilon}^{0.06}$)	$E = 6227 \cdot \rho^3 \cdot \dot{\epsilon}^{0.06}$	0.66	human tibia
	Enns-Bray WS. ⁹⁵	power function ($E = a \cdot \rho^b \cdot DA^c \cdot \dot{\epsilon}^d$)	$E = 20290 \cdot \rho^{0.63} \cdot DA^{0.0099} \cdot \dot{\epsilon}^{-0.55}$	0.79	bovine femurs and tibiae
Strength, σ_u (MPa)	Carter DR. ⁹⁶	Power Function ($\sigma_u = a \cdot \rho^b \cdot \dot{\epsilon}^c$)	$\sigma_u = 68 \cdot \rho^2 \cdot \dot{\epsilon}^{0.06}$	-	human tibia and bovine femurs
	Linde F. ⁹⁴ _1	Power Function ($\sigma_u = a \cdot \rho^b \cdot \dot{\epsilon}^c$)	$\sigma_u = 40.2 \cdot \rho^{1.65} \cdot \dot{\epsilon}^{0.073}$	0.93	human tibia
	Linde F. ⁹⁴ _2	Linear Density ($\sigma_u = (a + b \cdot \rho) \cdot \dot{\epsilon}^c$)	$\sigma_u = (-5.8 + 37.1 \cdot \rho) \cdot \dot{\epsilon}^{0.073}$	0.93	human tibia
	Linde F. ⁹⁴ _3	Quadratic Density ($\sigma_u = a \cdot \rho^2 \cdot \dot{\epsilon}^c$)	$\sigma_u = 52.1 \cdot \rho^2 \cdot \dot{\epsilon}^{0.070}$	0.92	human tibia
	Linde F. ⁹⁴ _4	Linear Strain Rate ($\sigma_u = \rho^b \cdot (a + c \cdot \dot{\epsilon})$)	$\sigma_u = \rho^{1.53} \cdot (25.0 + 2.26 \cdot \dot{\epsilon})$	0.93	human tibia
	Linde F. ⁹⁴ _5	power function ($\sigma_u = a \cdot \rho^2 \cdot \dot{\epsilon}^{0.06}$)	$\sigma_u = 51.5 \cdot \rho^2 \cdot \dot{\epsilon}^{0.06}$	0.92	human tibia
	Enns-Bray WS. ⁹⁵	power function ($\sigma_u = a \cdot \rho^b \cdot DA^c \cdot \dot{\epsilon}^d$)	$\sigma_u = 24.1 \cdot \rho^{1.07} \cdot DA^{0.83} \cdot \dot{\epsilon}^{0.11}$	0.87	bovine femurs tibiae*
	Shim VPW. ⁹⁷	Power Function ($\sigma_u = a \cdot \rho^b + c \cdot \dot{\epsilon}^d$)	$\sigma_u = 5.09 \cdot \rho^{2.35} + 0.545 \cdot \dot{\epsilon}^{0.06}$	-	human vertebrae
Shear modulus, G (MPa)	Kasra M. ⁹²	Power Function ($G = a \cdot \rho^b \cdot \dot{\epsilon}^c$)	$G = 595.5 \cdot \rho^{1.08} \cdot \dot{\epsilon}^{0.07}$	0.84	sheep vertebrae

*DA is the degree of anisotropy, assumed to be 1.35 by the authors

3.3. Statistical analysis

The statistical methods were developed in consultation with the KU Leuven Biostatistics and Statistical Bioinformatics Centre. The dataset had a three-level hierarchical structure representing 84 zone observations, from 28 VB punches nested within 4 subjects (Figure 2). To account for subject-specific variability and the hierarchical structure of the data, nested random intercept linear mixed-effects models (LMMs) (Equation 8) were used with restricted maximum likelihood (REML). The models were implemented in R 4.5.3 (R Core Team, 2026) using the lme4 package⁹⁸ and the full reproducible code is available in the GitHub repository⁹⁹. Each measured value (tortuosity, porosity, and mean pore radius) was included as the dependent variable and zone was included as a fixed effect with two levels: central and peripheral. Because multiple zone measurements were obtained from each punch and multiple punches were sampled from each subject, random intercepts were included for subjects and for punches nested within the subject. Fixed effects were tested using Satterthwaite's method, and the estimated effect size, standard error (SE), 95% confidence interval (95% CI), and p-value were reported for the fixed effects.

Statistical power for the tortuosity, porosity, and mean pore radius analysis was estimated using a Monte Carlo simulation approach⁹⁸ based on the fitted LMM. For each simulated dataset, the significance of the zone effect was assessed by refitting the model using restricted maximum likelihood (REML) and obtaining p-values via Satterthwaite's method. Power for the observed experimental design was estimated as the proportion of simulations ($n = 500$) in which a significant zone effect was detected ($p < 0.05$). Normality was assessed for sufficiently powered models, as tests of normality are unreliable in underpowered ones. Power curves were then generated by repeating this procedure across expanded designs varying the number of subjects under balanced and unbalanced BEP sampling while preserving the observed within-subject hierarchical structure. The number of subjects required to achieve at least 80% power was identified, providing guidance for planning larger future studies.

3.4. Verification using a ground truth object at the macro scale

To verify the accuracy of the workflow described above, an object with known ground truth properties was used. A triply periodic minimal surface (TPMS) scaffold with a primitive geometry

(Figure A.1) defined by the equation $\cos(x) + \cos(y) + \cos(z) = 0$ was 3D printed and scanned at a voxel resolution of 4 μm . The scaffold was CaP-based (calcium phosphate), 3D printed via vat polymerization in a layer-by-layer manner while exposed to ultraviolet (UV) light to initiate resin polymerization. The scaffold measured 6 mm in both diameter and height, and had a unit cell size of 2.19 mm, and a designed porosity of 77.5% and an as-manufactured porosity of $66.6\% \pm 5.5\%$, as previously described by Bouakaz *et al.*¹⁰⁰. The manufactured macrochannel diameters were measured in CTAnalyzer (CTAn, version 1.20.8.0, Bruker microCT, Kontich, Belgium) using the built-in measuring tool by drawing linear distances across the pores, where the largest channels measured 2 mm.

In this context, it is important to distinguish between two types of voids present in the printed scaffold. The intended scaffold pores, being the macrochannels defined by the TPMS design, have characteristic diameters at the millimeter scale. Manufacturing variability can introduce additional pores at the micrometer scale, for example, through surface roughness or local defects. These micropores are not part of the functional scaffold architecture and cannot be reliably measured by manual methods. As a result, the verification focused on the macrochannels, which are the relevant structural features and can be measured consistently. This verification supports the geometric performance of the workflow under controlled conditions. However, it does not fully establish segmentation accuracy in heterogeneous biological bony endplate tissue.

4. Results

4.1. The semi-automated workflow was verified against ground truth objects

The workflow reported a maximum channel diameter of 1.9 mm, corresponding to an accuracy of 95% compared to the results obtained with established tools such as CTAn. The calculated porosity was 71%, representing a porosity accuracy of 93.4% relative to the designed value. This value lies within the as-manufactured range and differs by only 0.8 standard deviations from the reported mean.

4.2. Porosity varies between central and peripheral regions, while pore geometry remains constant

Percolation was observed in 39 of the 84 samples. Among the evaluated regions, 54% of central zones and 43% of peripheral zones exhibited percolation. Zones without percolation had undefined tortuosity and were excluded from subsequent tortuosity analysis (Figure A.2).

The estimated effect of the zone on tortuosity (Figure 4a) was not statistically significant ($p = 0.441$). The model effect difference was -0.3403 (95% CI: -1.2519 to 0.5712 , SE of the difference = 0.4369), corresponding to mean tortuosity values of 4.5650 ± 0.51 (SE) in the central zone and 4.9053 ± 0.46 (SE) in the peripheral zone. The zone explained only 1.6% of the total variability, subject-to-subject variability accounted for 23.1%, differences between VB punches within the same subject accounted for 0%, and residual variance accounted for the remaining 75.3%.

Porosity (Figure 4b) differed significantly between zones ($p < 0.001$). The model effect difference was 0.0669 (95% CI: 0.0311 to 0.1026), with a low standard error of the difference (SE = 0.0179) indicating high precision. The estimated mean porosity was 0.3746 ± 0.05 (SE) in the central zone and 0.3077 ± 0.05 (SE) in the peripheral zone. The zone explained only 4.6% of the total variability, differences between punches within the same subject accounted for 46.8%, subject-to-subject variability accounted for 21.6%, and residual variance accounted for the remaining 27.0%. The distribution of standardized residuals indicated that the porosity data satisfied the assumption of normality.

Skeletonization of the BEP zones revealed a highly complex pore network. The extracted centerlines contained an average of 4,810 anchors per sample, with a minimum of 575 anchors in any given zone. Each anchor represents a local geometric inflection point, indicating a change in pore direction or radius. The effect of zone on mean pore radius (Figure 4c) was not statistically significant ($p = 0.2$). The model effect difference was $-1.728 \mu\text{m}$ (95% CI: -4.4 to $0.94 \mu\text{m}$, SE of the difference = $1.33 \mu\text{m}$), corresponding to estimated mean radius of $19.37 \pm 2.49 \mu\text{m}$ (SE) in the central zone and $17.64 \pm 2.37 \mu\text{m}$ (SE) in the peripheral zone. The zone explained only 0.6% of the total variability, differences between punches within the same subject accounted for 58.2%, subject-to-subject variability accounted for 10.3%, and residual variance accounted for the remaining 30.8%.

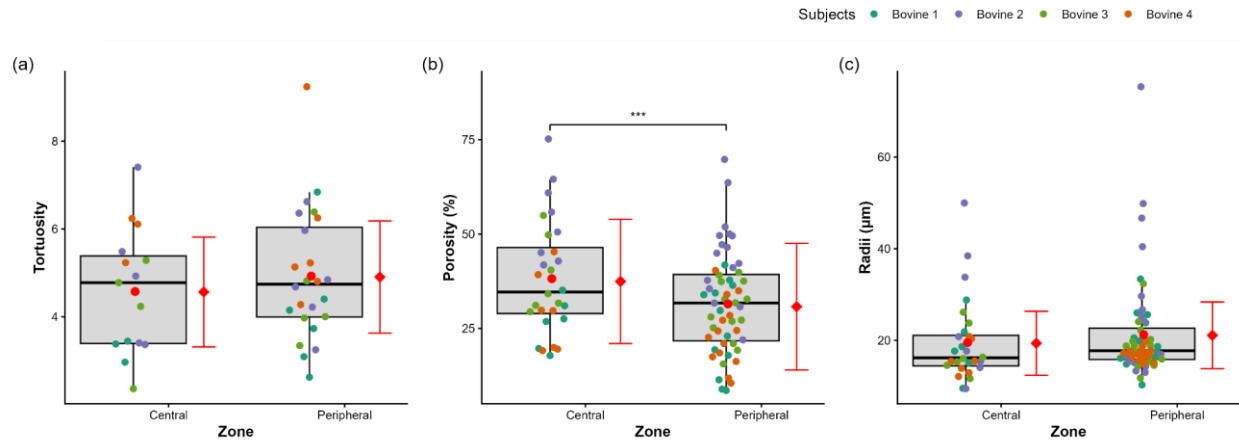


Figure 4: boxplot of the raw data showing the interquartile range (IQR), mean (red circle), median (horizontal line), and the $1.5 \times \text{IQR}$ (whiskers) of the regional morphological properties in the BEP. The red diamonds and error bars represent model-based estimates from linear mixed-effects models. Regional differences between central and peripheral zones in tortuosity (a), porosity (b), and mean pore radius (c). Central and peripheral zones are not independent observations and their statistical significance is based on the LMM. $P < 0.001$ is indicated by ***.

Under the observed effect size and variance structure, and based on 500 simulations at $\alpha = 0.05$, the estimated tortuosity statistical power of the current design was 0.14 (95% CI 0.11–0.18), for porosity it was 0.96 (95% CI 0.94–0.97) and for the mean pore radius it was 0.25 (95% CI 0.21–0.29).

In simulations varying the number of subjects while preserving the observed within-subject sampling structure, for unbalanced designs, with an average of 7 BEPs per subject (Figure A.3) similar to the current design, power increased gradually until it reached 0.80 with 55 subjects for tortuosity and 0.88 with 22 subjects for mean pore radius. Balanced designs would require fewer subjects, for example, with exactly 7 BEPs per subject, power reached 0.85 with 30 subjects for tortuosity and 0.82 with 20 subjects for mean pore radius (Figure A.4).

4.3. Not all BEP pores are interconnected, which affects predicted permeability

Considering only percolating pores, the predicted mean intrinsic permeability was $3.58 \text{ D} \pm 3.99 \text{ D}$ at the central zones and $3.18 \text{ D} \pm 3.03 \text{ D}$ for the peripheral zones (Figure 5).

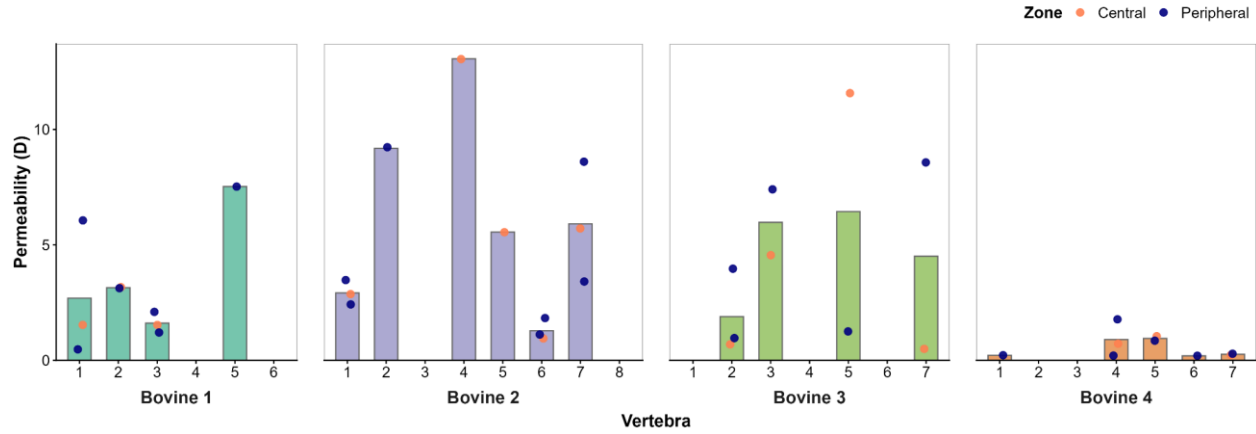


Figure 5 Differences in intrinsic permeability of the BEP at each punch. The average permeability of each BEP in each punch in each subject is represented by a grey bar with navy blue dots representing peripheral zone values and orange dots representing central zone values. The x-axis values represent identifiers assigned to the punches and do not correspond to the vertebral level.

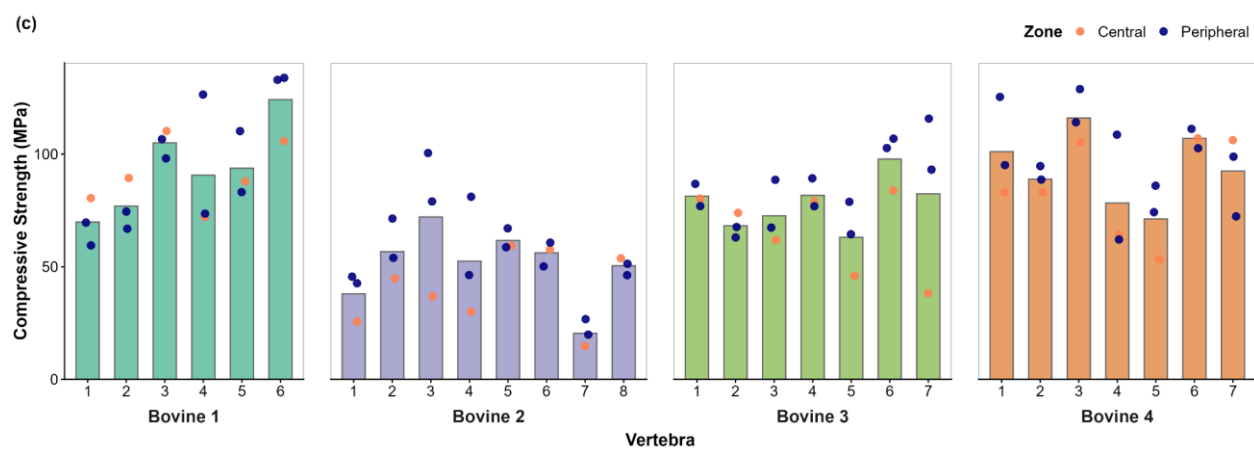
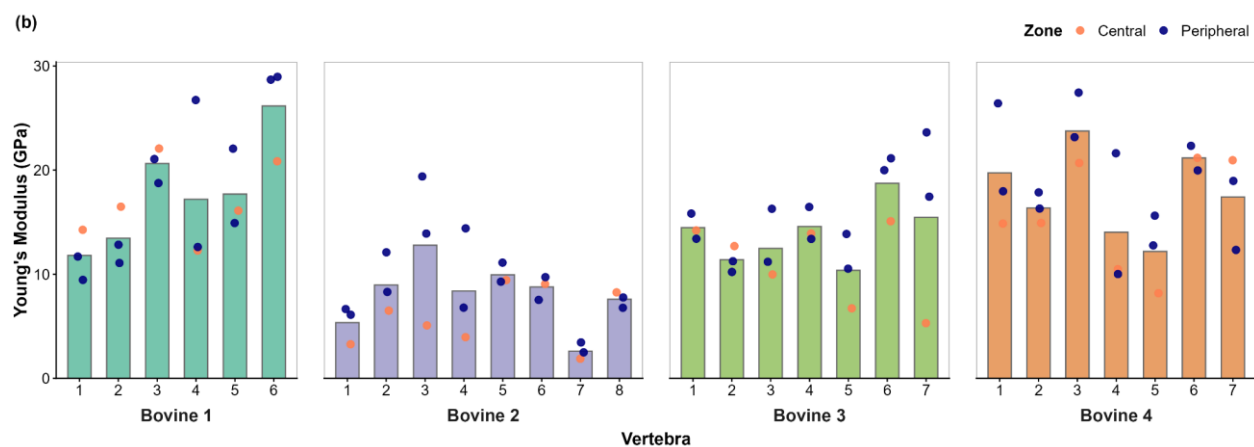
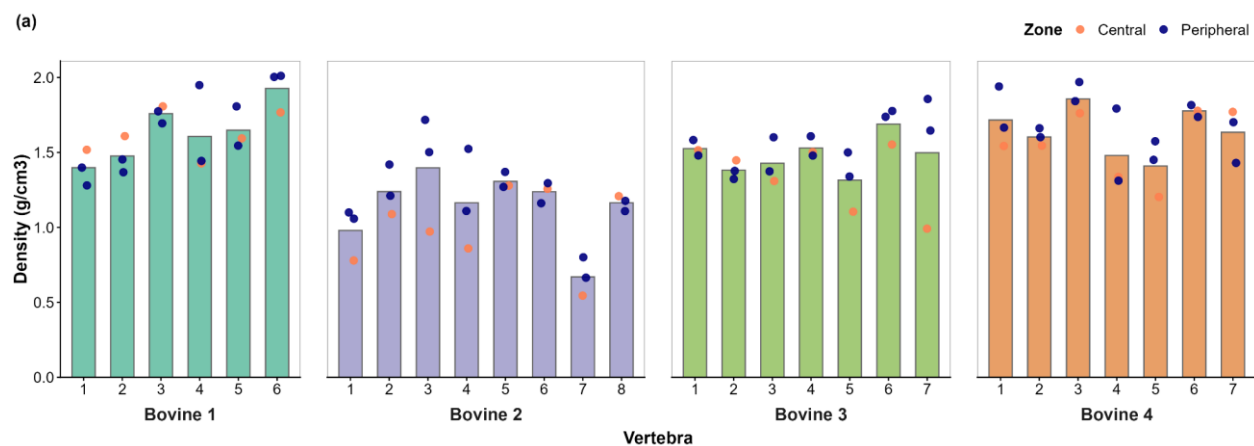
4.4. Inferred density and mechanical properties of the BEP are zone-dependent

To predict mechanical properties from these morphological measures, multiple mathematical models were used in this study. To consolidate these results into a single predictive framework, 3D regression models for Young's modulus and compressive strength were generated by fitting continuous surfaces over the density-strain rate space (Figure A.5). These surfaces provide continuous predictions of Young's modulus E and compressive strength σ_u as functions of both density and strain rate, with coefficients of determination (R^2) of 0.95 and 0.99, respectively.

$$E = 4388.03\rho^{2.65} \cdot \dot{\epsilon}^{-0.08} + 1010.68 \quad (8)$$

$$\sigma_u = 35.33\rho^{1.87} \cdot \dot{\epsilon}^{0.07} + 3.4 \quad (9)$$

The predicted mean central and peripheral densities of the BEP were $1.36 \text{ g/cm}^3 \pm 0.33 \text{ g/cm}^3$ and $1.51 \text{ g/cm}^3 \pm 0.29 \text{ g/cm}^3$ respectively (Figure 6a). The predicted Young's modulus was $12.09 \text{ GPa} \pm 5.9 \text{ GPa}$ for the central zone and $15.04 \text{ GPa} \pm 6.45 \text{ GPa}$ for the peripheral zone (Figure 6b) while the predicted compressive strength was $69.07 \text{ MPa} \pm 26.67 \text{ MPa}$ for the central zone and $81.74 \text{ MPa} \pm 26.49 \text{ MPa}$ for the peripheral zones (Figure 6c), and the predicted shear modulus was $0.83 \text{ GPa} \pm 0.21 \text{ GPa}$ for the central zone and $0.93 \text{ GPa} \pm 0.19 \text{ GPa}$ for the peripheral zone (Figure 6d).



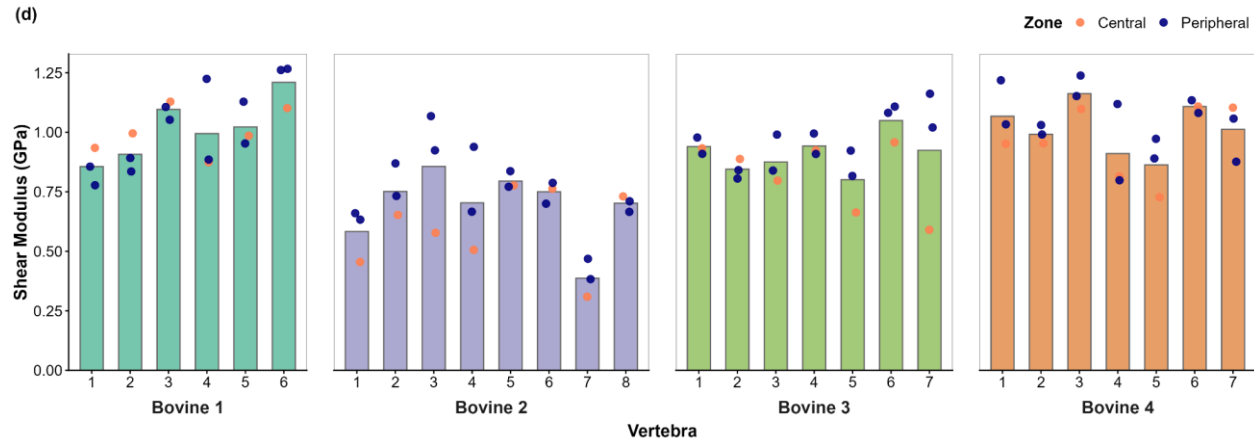


Figure 6 Density (a) of the BEP at each punch and the predicted mechanical properties: Young's modulus (b), compressive strength (c), and shear modulus (d). The average value of the density or mechanical property for each punch (numbers on x-axis) in each subject (Bovine 1-4) is represented by a grey bar with navy blue dots representing peripheral zones and orange dots representing central zones. The x-axis values represent punch identifiers and do not correspond to the vertebral level.

5. Discussion

This study introduces a semi-automated pipeline for quantitative characterization of BEP microarchitecture, providing a workflow to quantify porosity and pore morphology in BEP samples, generate 3D mesh structures, and predict their mechanical and transport properties based on relationships amalgamated from the literature. This enables reproducible assessment across anatomical zones and provides a robust foundation for accurate modeling and simulation in vertebral applications. The workflow uses μ CT scans of Hexabrix-stained VB punches to enhance contrast between bone and surrounding soft tissues. The tool was verified using a ground truth TPMS scaffold with a primitive geometry, achieving 95% and 93.4% accuracies for radius and porosity measurements, respectively, comparable to the standard tools in CTAn. These results reflect the tool's performance on the intended geometric features rather than defects or manufacturing-induced micropores. They suggest that the workflow reliably captures the macrostructural characteristics of a complex porous scaffold, which aligns with the scale and type of features quantified in the biological samples.

BEP pores vary widely in the literature, from a few micrometers up to several hundred micrometers. In humans, the most commonly reported average pore diameter ranges from approximately 20 to

60 μm ^{101–105}. Data for bovine BEPs are more limited, with reported pore diameters ranging from 15 to 84 μm ¹⁰⁶. One study directly compared bovine and human BEPs using imaging measurements and found the average diameters to be 33 μm and 58 μm , respectively¹⁰¹. In the present study, we differentiated between central and peripheral zones, and the average pore diameters of bovine BEPs were 38.74 μm and 35.28 μm in the central and peripheral zones, respectively. No significant difference was observed between the zones, which is consistent with the reported values.

Not all pores within the zones were true percolating pores, as some were disconnected blind pores that terminated within the tissue, or lateral pores that terminated at the zone sides. This could be the result of the specific zone selected for analysis, as previously mentioned, and they were excluded from the tortuosity and permeability calculations. Percolation was detected in less than half of the samples and varied between zones, with 54% of central zones and 43% of peripheral zones exhibiting connectivity. Tortuosity did not differ significantly between central and peripheral zones, but there was a noticeable subject-to-subject variability (23.1%), whereas differences between punches within the same subject were negligible. This suggests that inter-subject differences have a stronger influence on tortuosity than regional pore network geometries. This may be partly due to the exclusion of zones where tortuosity was undefined, but given that the study was underpowered for this parameter, the results should be interpreted with caution pending confirmation in larger cohorts.

Porosity, in contrast, exhibited significant spatial variation ($p < 0.001$), with central zones showing higher mean porosity than peripheral zones. Although zone location accounted for only 4.6% of the variability, differences between punches within the same subject contributed 46.8%, and subject-to-subject variability accounted for 21.6%. Since this study was sufficiently powered for this parameter, the observed variation in porosity can be confidently attributed to both intra-subject and inter-subject heterogeneity as dominant contributors.

Analysis of mean pore radius further emphasized the importance of intra-subject variability. Although the mean pore radius did not differ significantly between central and peripheral zones, differences between punches within the same subject accounted for 58.3% of the variability. Inter-subject differences were comparatively smaller (10.3%), indicating that the local geometry of individual pores could be largely determined by the vertebral level. These results should also be interpreted with caution pending confirmation in larger cohorts given that the study was

underpowered for this parameter. The high number of average anchor points in the skeletonized BEPs (representing local geometric inflection points, indicating changes in pore direction or radius) also supports the notion that the BEP is not a homogeneous structure but instead exhibits considerable spatial and morphological variability throughout. Collectively, these results suggest that the BEP exhibits both spatial heterogeneity (central vs peripheral regions) and pronounced inter-sample and inter-subject differences dominating porosity and pore size characteristics, while tortuosity is influenced more by inter-subject variability.

As previously mentioned, one of the possible applications of the presented pipeline is increasing the level of sample-specificity in numerical simulations currently used to interpret experimentally obtained organ culture results⁷. The presented pipeline can predict mechanical and transport properties based on the obtained morphological parameters, which in the case of porosity have been shown here to vary significantly across and within subjects. Considering only the percolating zones, the intrinsic permeability of the BEP was predicted to be higher at the central zones compared to the peripheral zones. The predicted density of the BEP, $1.36 \text{ g/cm}^3 \pm 0.33 \text{ g/cm}^3$ and $1.51 \text{ g/cm}^3 \pm 0.29 \text{ g/cm}^3$ for the central and peripheral zones, respectively, align with values reported in the literature, $0.75\text{-}2.1 \text{ g/cm}^3$ ^{107–110}.

The bovine model was selected in this study because it is widely used in intervertebral disc organ culture and endplate-related experimental workflows, it is readily available as fresh tissue, and it allows reproducible sample collection with controlled processing^{47–51}. These practical and methodological advantages were important for the imaging-to-quantification pipeline. At the same time, we acknowledge that bovine coccygeal segments are not the primary load-bearing spinal levels and are exposed to a different mechanical environment than human lumbar vertebrae. Because endplate microarchitecture adapts to mechanical demand, differences in loading history may influence porosity, connectivity, and pore orientation, and therefore the absolute values of predicted mechanical and transport properties. Accordingly, the present results should be interpreted as specific to the bovine BEP samples analyzed in this study. This, however, does not take away from the main objective of this study, which is demonstrating the semi-automated pipeline for analysis of μ CT images of the BEP, and the illustration of how the proposed pipeline can be applied within organ culture workflows to derive sample-specific mechanical and transport properties to inform specimen-specific loading or fluid flow simulations.

Biological segmentation remains challenging due to partial-volume effects, heterogeneous mineralization, and less distinct tissue interfaces. The TPMS-based verification performed in this study primarily demonstrates the robustness of the pipeline for detecting geometric segmentation under the assumption of uniform tissue mineralization. Limited biological replicates ($n=4$) reduced the statistical power to detect zone effects for tortuosity and mean pore radius, implying that the absence of statistical significance does not rule out biological zone effects on these parameters. Future work should therefore integrate this imaging pipeline with direct mechanical and permeability measurements on biological specimens, using human lumbar sections if feasible, to validate and refine the approach. Additionally, combining this pipeline with computational models of the CEP and the nucleus pulposus could enable a more comprehensive assessment of the mechanical and transport properties of the IVD as a whole.

The BEP plays a critical dual role in both mechanical load regulation and nutrient transport to the intervertebral disc, functions that depend strongly on its pore geometry and connectivity. Even small increases in porosity can substantially reduce local bone strength and elasticity while also influencing solute diffusion. Accurate characterization of this microarchitecture is therefore essential for predicting mechanical competence, permeability, and disc health. The semi-automated processing pipeline presented here enables efficient, reproducible characterization of BEP microarchitecture. This pipeline provides sample-specific morphological values that can be used as inputs for computational simulations of vertebral BEP function. The segmented BEP mesh can be directly imported into a computational workflow where the quantified morphological, mechanical, and transport properties replace the idealized or literature-averaged parameters that are currently typically used. Grounding these numerical models in empirically derived structural parameters would enable more accurate predictions of stress transfer, strain localization, and load-bearing capacity by capturing the true heterogeneity of the BEP microstructure in mechanical simulations. Additionally, the predicted permeability and measured pore architecture would reliably inform solute diffusion and fluid flow models, thereby improving computational predictions of nutrient transport to the intervertebral disc in transport simulations. Disc organ culture under bioreactor conditions would particularly benefit from integrating this tool into the experimental workflow. Incorporating sample-specific characterization allows investigators to explicitly account for the variability in inferred properties of the BEP, which is experimentally

515 difficult to fully separate from the CEP⁷. Hence, this workflow represents a step toward more
516 realistic *in vitro* and *in silico* modeling of vertebral endplate functions.

6. Author contributions:

AA, JN, and LG conceptualized the study. AA conducted major literature research, developed the codes, acquired the CT scans, created the visualizations, and wrote the main manuscript. GN contributed to building the Docker computing environment and developed the initial scripts for CT-based sample identification. KC contributed to isolating IVDs and to acquiring the CT scans. GN, KC, and BG contributed to reviewing the manuscript. JN and LG contributed to writing, analyzing the results, and providing funding support. All authors read and approved the final version of the manuscript.

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8. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix

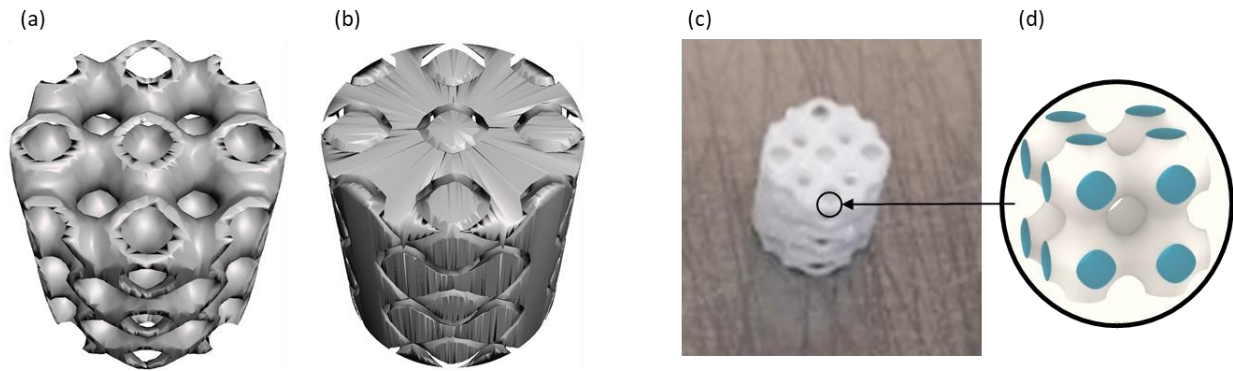


Figure A.1 Triply periodic minimal surface (TPMS) scaffold with primitive geometry used for verification of the workflow: designed structure defined by the equation $\cos(x) + \cos(y) + \cos(z) = 0$ (a), the negative structure illustrating interconnected macrochannels (b), the as-manufactured 3D-printed CaP-based structure (c), and corresponding repeating unit cell of the scaffold (d).

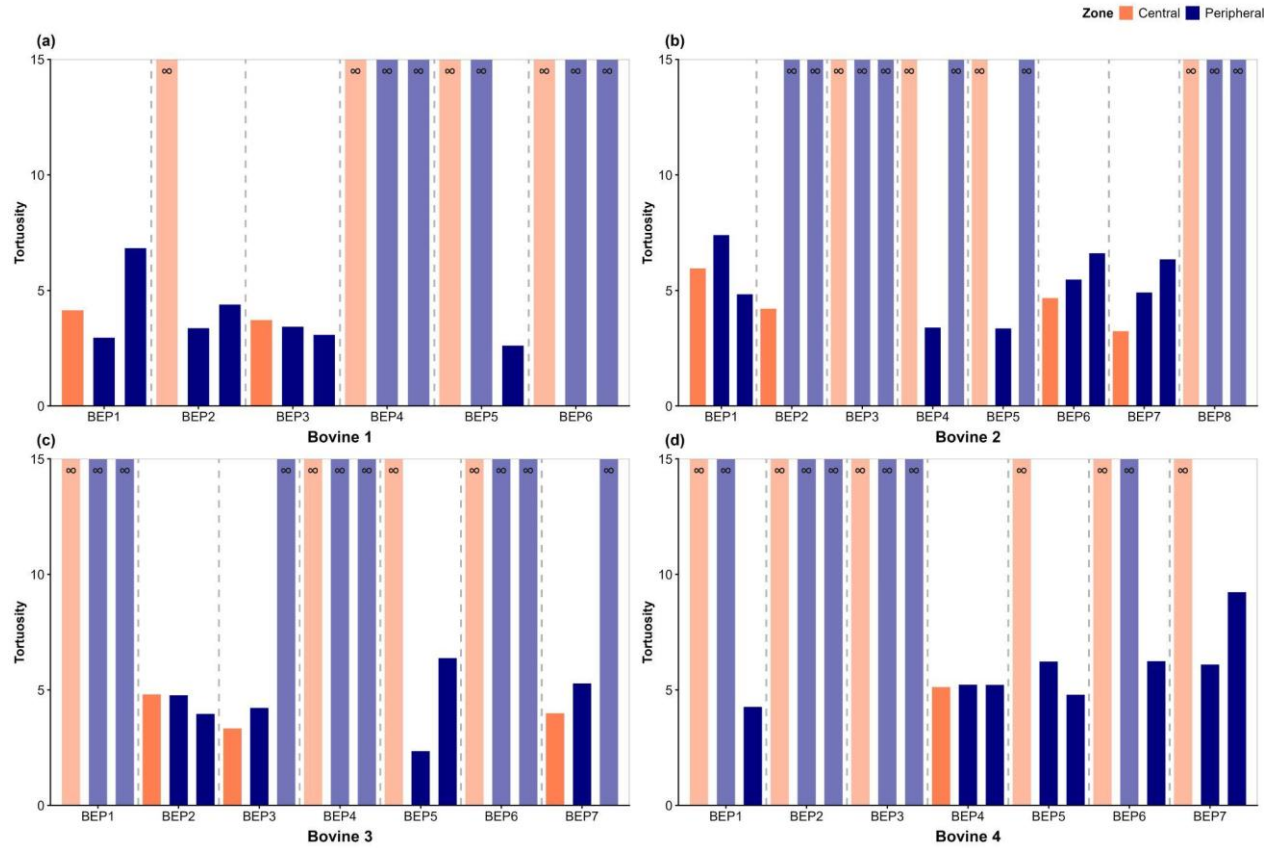


Figure A.2 Difference in tortuosity between the central and the two peripheral zones in each BEP punch in bovine 1 (a), bovine 2 (b), bovine 3 (c), and bovine 4 (d). The translucent bars represent infinite tortuosity, indicating non-percolating zones. The numbers on the x-axis refer to the VB punch but do not refer to the vertebral level. The x-axis values represent arbitrary identifiers assigned to BEP punches and do not correspond to vertebral level.

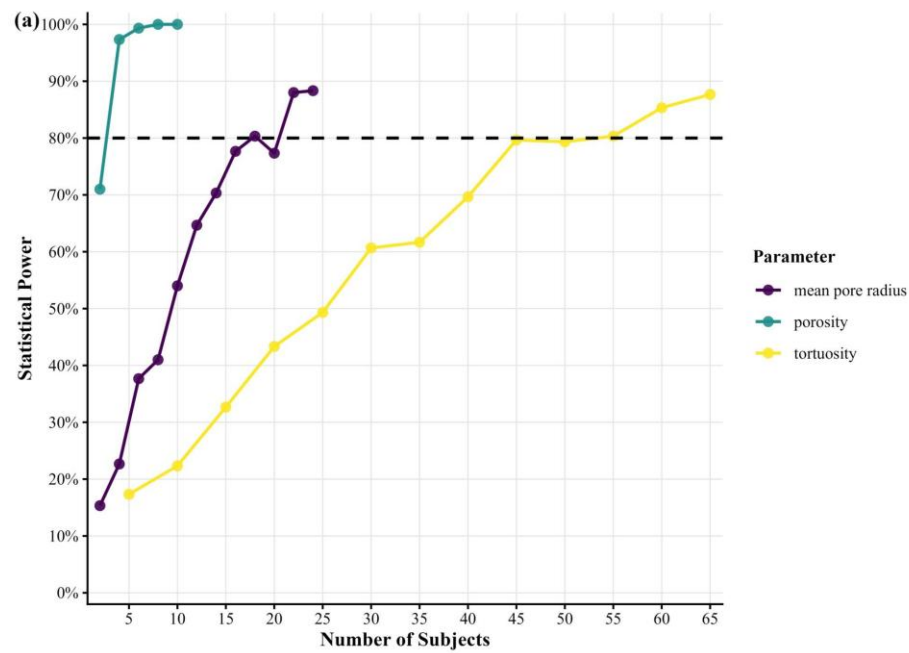
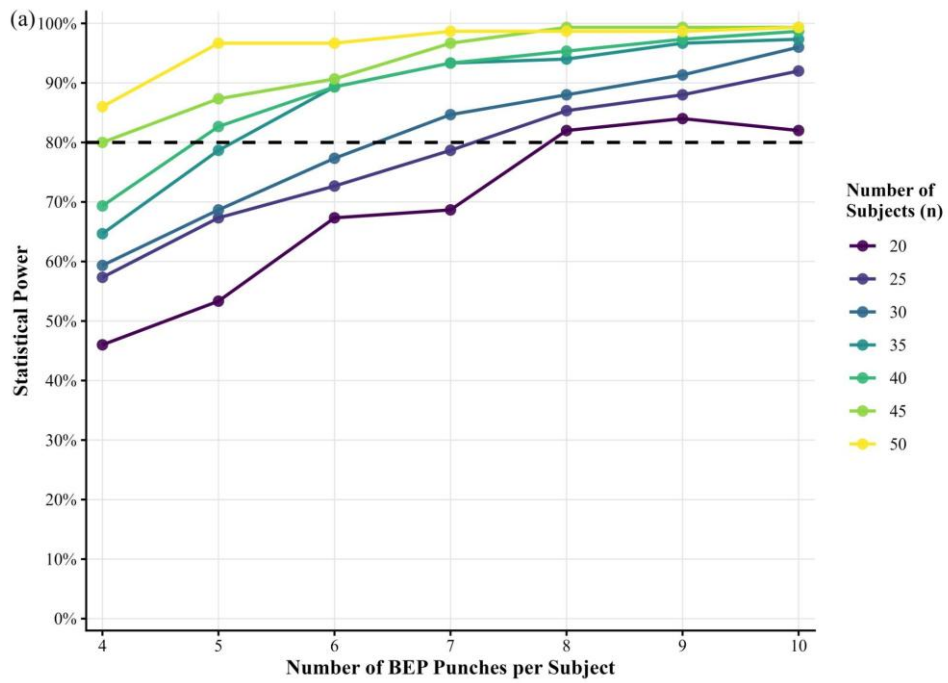
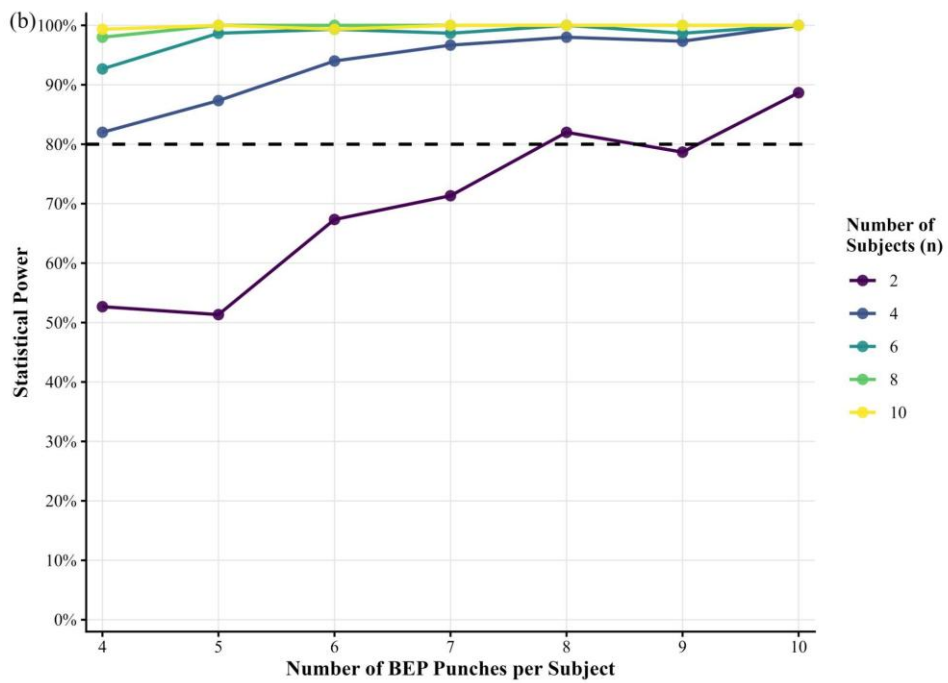


Figure A.3 Power Analysis for unbalanced designs: Effect of the number of biological replicates (subjects) with an average of 7 BEPs per subject on the statistical power for LMM of tortuosity (a), porosity (b), and mean pore radius (c).



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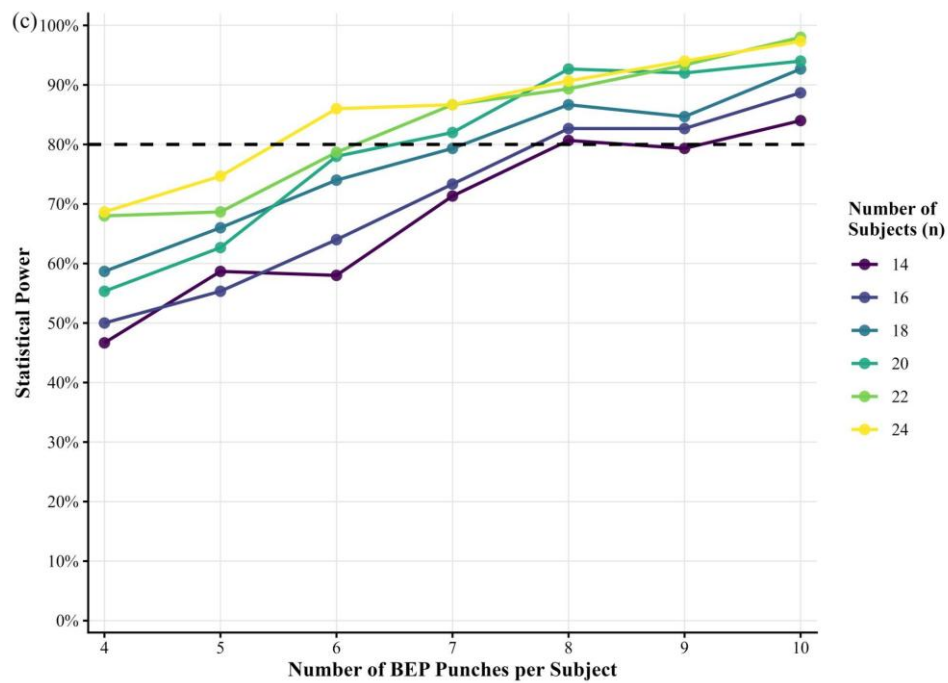


Figure A.4 Power Analysis for balanced designs: Effect of the number of biological replicates (bovine subjects) and of BEP punches per subject on the statistical power for LMM of tortuosity (a), porosity (b), and mean pore radius (c).

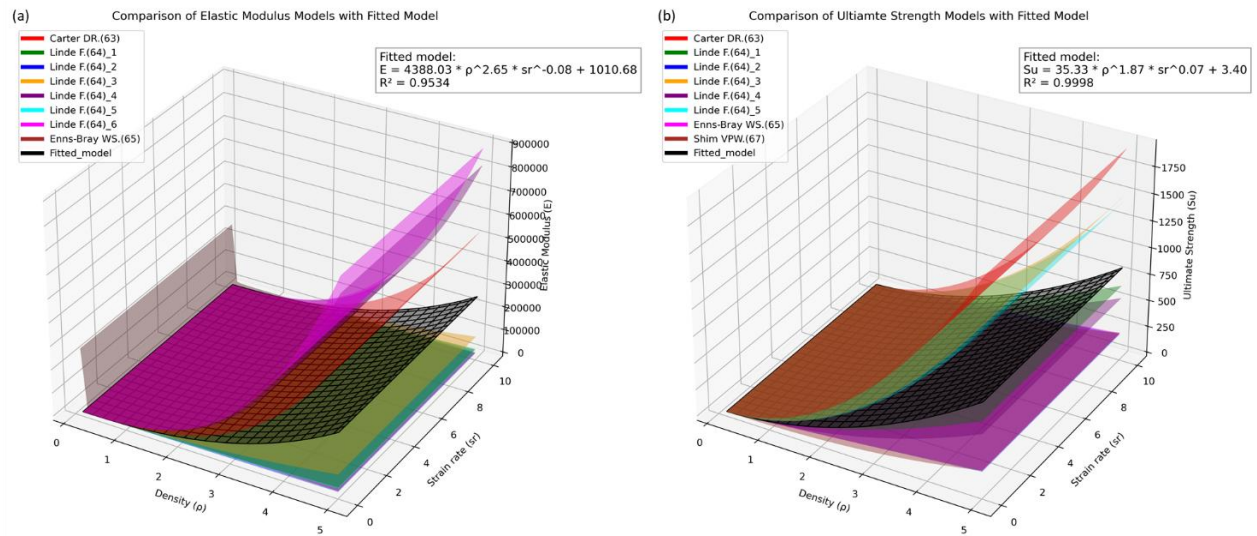


Figure A.5 Three-dimensional regression models using nonlinear curve fitting of the reported mathematical relationships between geometric properties and bone mechanical properties in literature to predict the corresponding Young's modulus (a) and compressive strength modulus (b).