Supplementary Information

Lengths:

Figure S1: The average length of axolotls is not significantly different between the timepoints.

Fitting Error:

The average error between experimental peak force and simulated peak force was not significantly different between the groups (p>0.05). There is no difference between the average error between experimental and simulated equilibrium force between groups (p>0.05) either.

Figure S2: (a)The average error between experimental peak force and simulated peak force (b) The average error between experimental equilibrium force and simulated equilibrium force

Proximal-Distal Differences 41 DPA:

Figure S3: 41 DPA (a) Instantaneous shear Modulus (b) Equilibrium shear Modulus (c) Relaxation Time Constant τ₁ (d) Relaxation Time Constant τ₂

Viscous Loss Parameters:

Figure S4: The viscoelastic loss is not significantly different between the proximal and distal indentation points in the 41 DPA sample.

Effect of Indenter Size and Choice of Model:

In this study, we aimed to investigate the effects of indenter size on normalized force for three different material models- poroelastic (PE), viscoelastic(VE), and poro-viscoelastic(BPVE), using finite element modeling as described in section 2.6. Soils type analysis was used for PE and BPVE models. The material parameters used in the study are listed in table S1. All samples underwent the same indentation procedure, with a consistent indentation depth (0.05 mm) and ramp time (1 s). The results of the study are presented in figure S5.

We found that when testing BPVE materials using indentation, the normalized force at small indenter sizes is not significantly affected by permeability. To accurately analyze and understand the influence of permeability on the peak force, micro/macro-sized indenters are necessary¹. This poses a challenge when trying to differentiate between the effects of permeability and viscoelasticity when analyzing indentation data from small specimens (with a surface area < 0.5mm x 0.5mm) using single indents. To address this issue, the use of larger indenters is not feasible. Instead, a two-term prony series viscoelastic model can be used to account for both fluid component and matrix relaxation effects when studying the behavior of such materials at smaller indenter sizes.

Given the small size of the samples and indenter diameter (0.3mm) used in our study, a two-term prony series viscoelastic model was deemed appropriate. This approach allows for a accurate analysis of the behavior using indentation, despite the challenges posed by small specimen sizes and the use of single indents.

Figure S5: Normalized force for three different models for the same indentation depth and ramp time.

Parameter	Description
Young's Modulus, E (MPa)	1.4
Poisson's ratio, v	0.25
Prony Series Time Constants, τ_1 , τ_2 (s)	20,120
Prony Series Shear Moduli, g1, g2	0.6, 0.2
Permeability, k (mm/s)	7.07 E-7
Void ratio	2.33
Dimensions (mm)	20x7

Table S1: Material parameters used for modeling

References:

1. Valluru PKR, Su A, Mehta S, Bajpayee A, Shefelbine S. Spatial and Temporal Mapping of Articular Cartilage Poro-Viscoelastic Material Properties Using Indentation. J Biomech Eng. Published online November 23, 2022:1-51. doi:10.1115/1.4056294