Feasibility of 3D Deformation and Strain Analyses by Micro-Computed Tomography

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BACKGROUND

- Measurement of strain in 3D is useful to assess the biomechanical function of tissues and materials [1]
- Micro-computed tomography (µCT) is an x-ray based imaging modality for determining 3D structures with micrometer (µm) level resolutions [2]
- Hydrogels can be created with a variety of material properties, but their deformation and strain behavior under load has previously been studied only in 1-D and 2-D [3]

HYPOTHESIS

µCT of hydrogels created with radiopaque particles can be analyzed under compression to assess 3D deformation and strain with high resolution.

OBJECTIVES

Create hydrogels with radiopaque particles and evaluate:

1. particle size suitable for visualization
2. axial and radial deformation and strain
   A. unconfined compression
   B. confined compression

METHODS

Samples Preparation

- Gel 3% agarose in Phosphate Buffered Saline (PBS) with 0.1% radiopaque particles
- Disks created: h=3mm, d=6.2mm (Table 1)
- Samples imaged with Skyscan1076 µCT at $(9\mu m)^3$ voxel size

Samples Compression

- Samples compressed to 30% over ~150s and 300s, respectively (Fig. 2AB)
- Samples equilibrated for 30 min than imaged in compressed state (Fig. 2CD)

Image Analysis

- 3D micro CT images thresholded to segment particles from background for multiple grayscale bin ranges
- 3D centroid position of individual particles determined through depth of gel, with z = 0 reference located at surface (Fig. 1 A1-3)
- Particle composition determined, with centroid variability between threshold bins
- For lowest variability particle size, axial and radial displacement of individual particles determined, comparing compressed position to initial position
- Displacement vs. position compared and fit to line, with strain being slope

Statistics

- Standard deviation of particle centroid position for each group
- Standard error for estimate of slopes from regressions ($p < 0.05$)
- Student's t-test of strain from regression slope and expected imposed strain ($p < 0.05$)

Experimental Group #	Particle size (um)	Background Particle Size (um)	Compression type
1	NA	None	Unconfined
2	80	20	
3	80	None	
4	40	20	
5	40	None	
6	80	20	Confined
7	80	None	
8	40	20	
9	40	None	

Table 1. Table of experimental groups tested with particle sizes, different background attenuation particles and compression type.

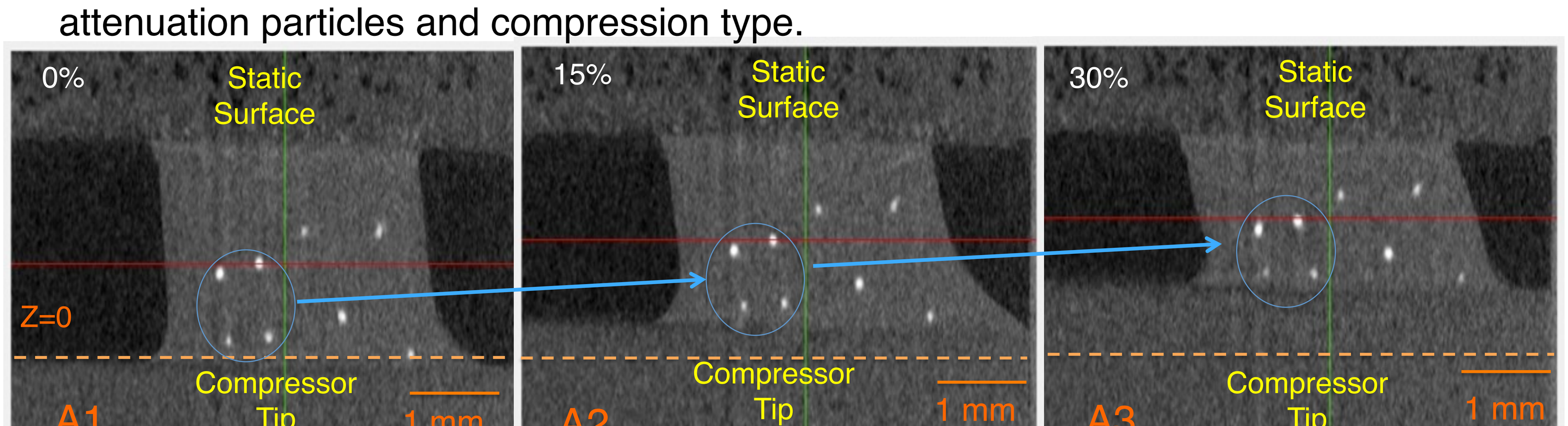

Figure 1. (A1-3) Imaging method of unique groups of particle tracking through 0, 15 and 30% compression (blue arrows).

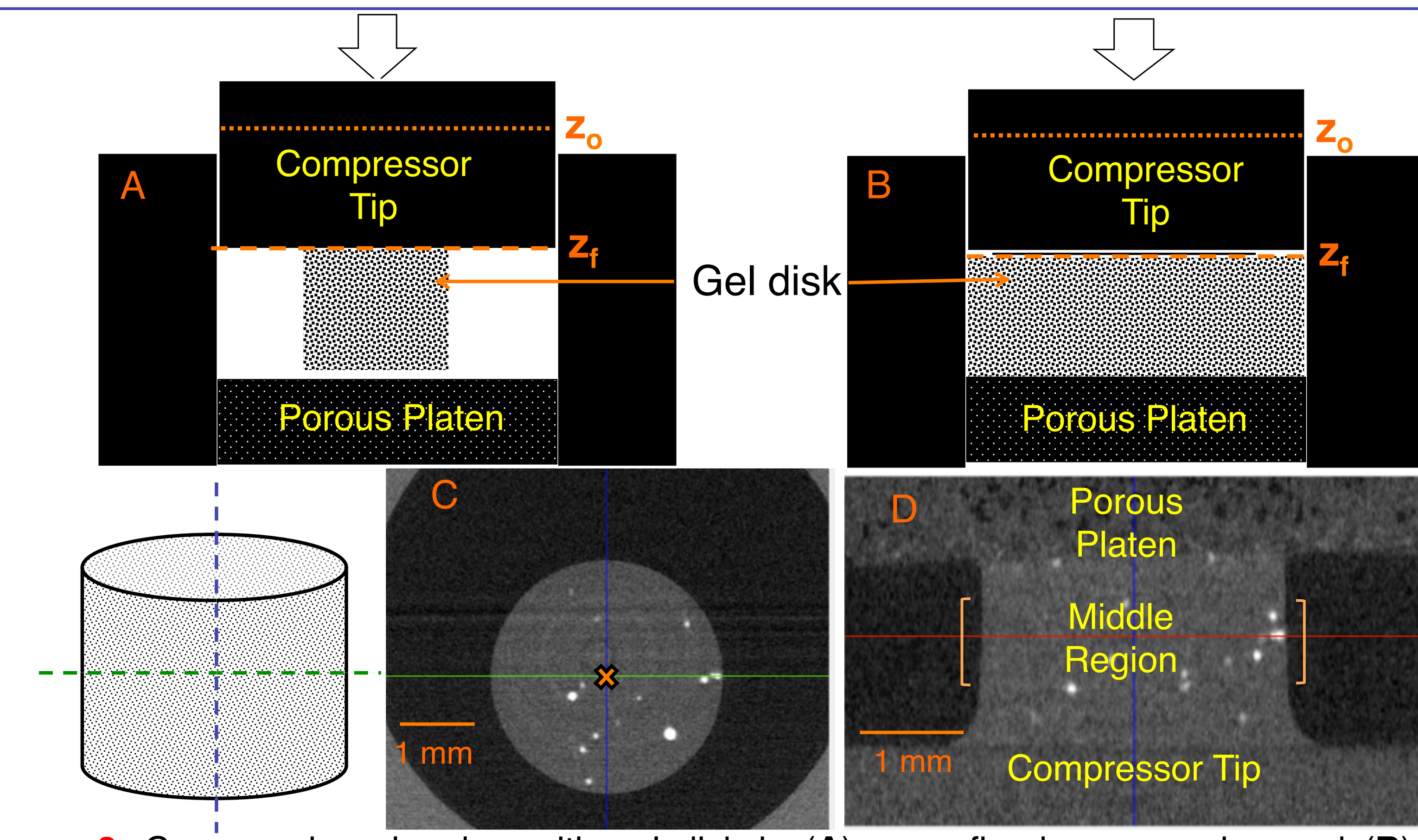

Figure 2. Compression chamber with gel disk in (A) unconfined compression and (B) confined compression. Cross section of hydrogel with the (C) green line showing the orange "X" marks center of the hydrogel used for radial analysis and (D) blue line the middle regions marks area used for radial analysis.

RESULTS

Particle Size and Attenuations

- Hydrogels containing 40µm particles approached micro CT resolution (2-4 pixels) and were excluded from further analysis, compared to the 80µm particles with much higher resolution (7-9 pixels) (Fig. 3AB)
- 80um particles showed smallest standard deviation of 0.616µm
- Adding 20µm particles increased background gel attenuation by 5.3% mm^{-1} relative to the 80µm particle hydrogel without the 20µm background

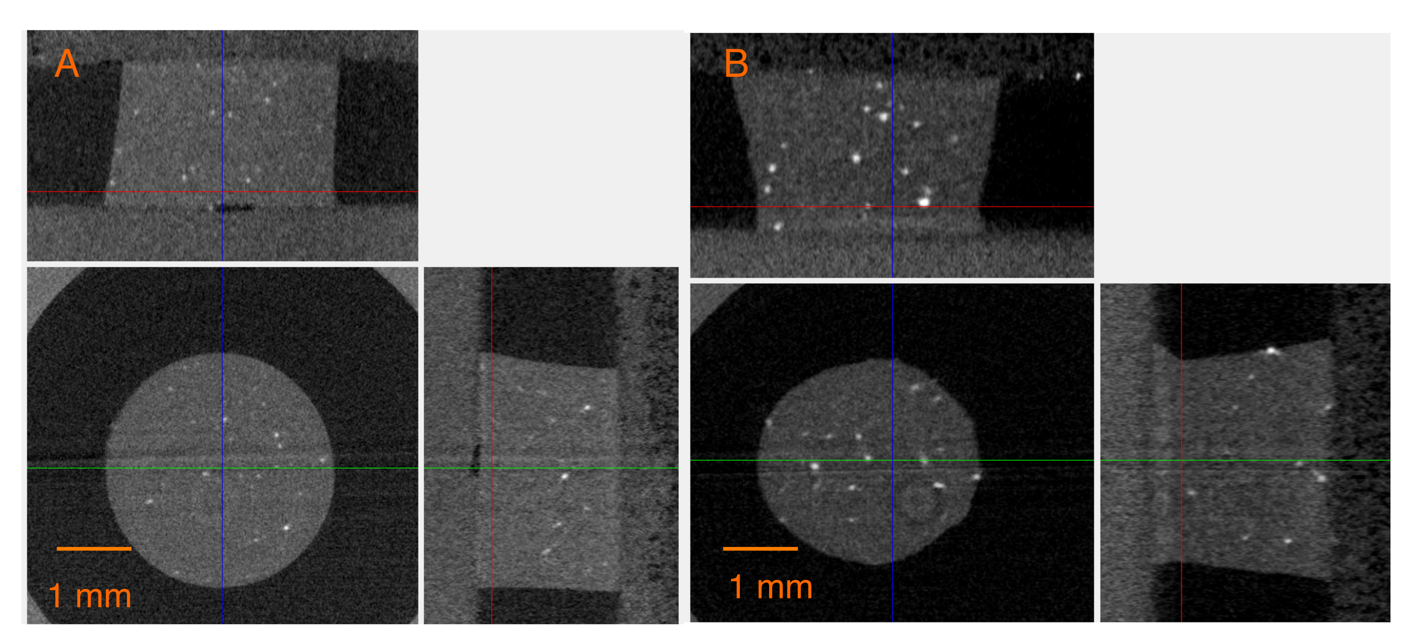

Figure 3. (A) 40µm particle hydrogel shown in orthogonal views and (B) 80µm particle hydrogel shown in orthogonal views.

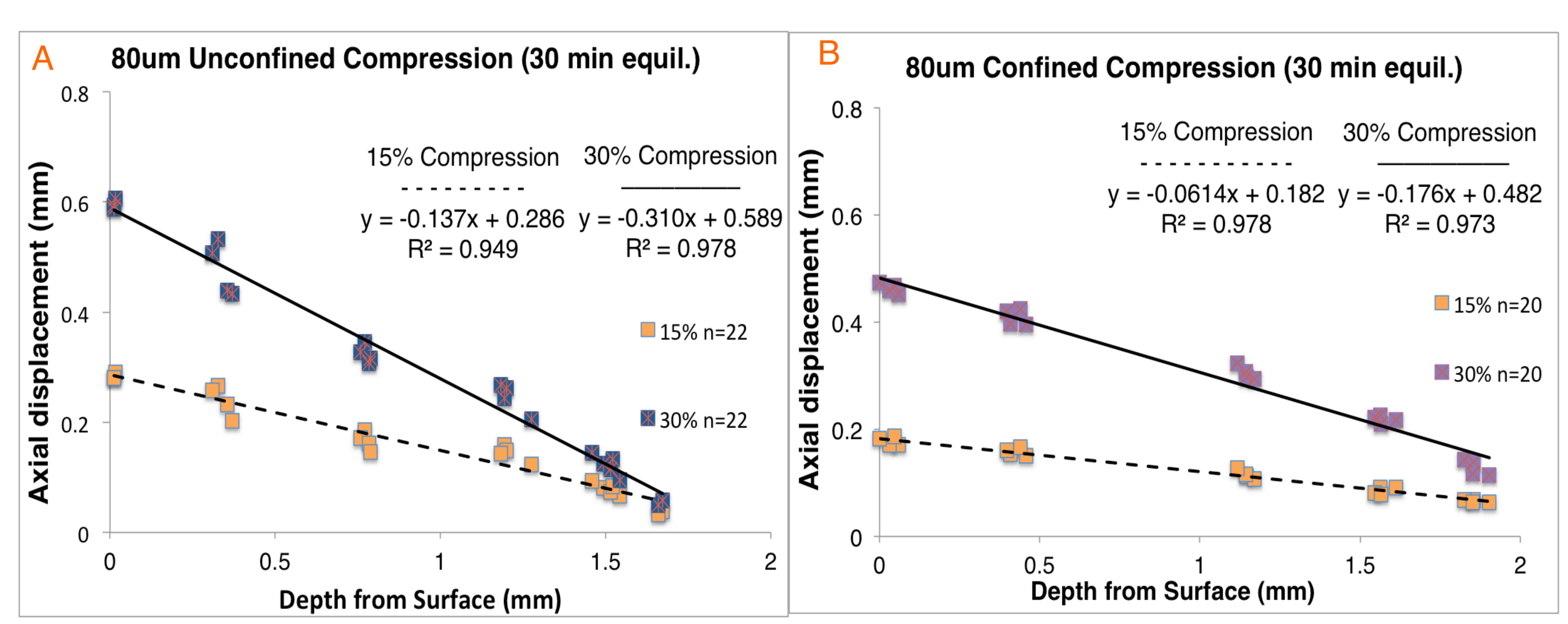

Figure 4. 80µm hydrogel strain measurements with 30 min. equilibration under (A) unconfined compression and (B) confined compression.

RESULTS (Cont.)

Axial and Radial Strain

- Linear regression of axial displacement vs. depth for 80um particles yielded a strain of 13.7% and 31.0% for 15% and 30% imposed unconfined compression, respectively (Fig. 4A)
- Linear regressions were statistically significant (p≈0, 0), R^2=0.95, 0.98
- Linear regression of axial displacement vs. depth for 80um particles yielded a strain of 6.1% and 17.6% for 15% and 30% imposed confined compression, respectively (Fig. 4B)
- Linear regression was statistically significant (p≈0, 0), R^2=0.98, 0.97
- Linear regression of radial displacement vs. radius showed low R^2 values of 0.45 and 0.47 for 15% and 30% compression, respectively, which were statistically insignificant (p=0.23, 0.23) (Fig. 5)

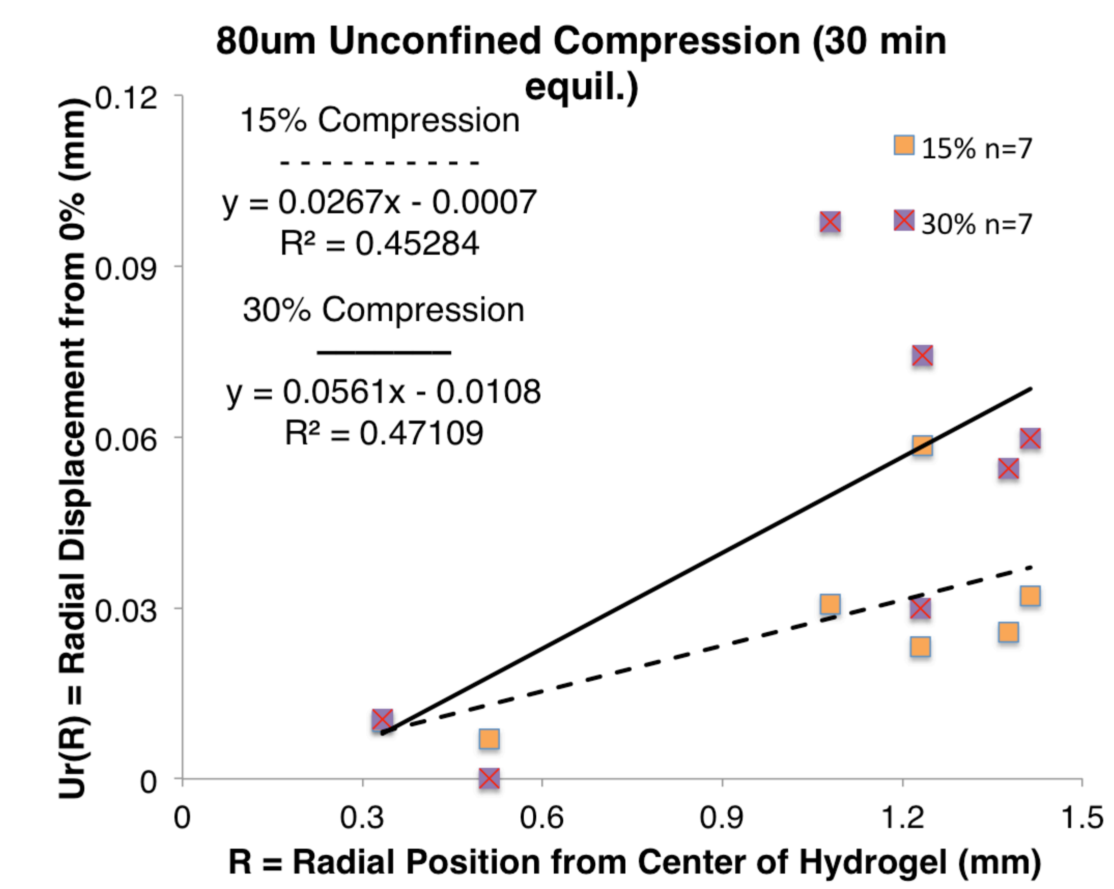

Figure 5. 80µm graph of radial strain with linear regression.

DISCUSSION

- 40µm size particles too small for tracking since they approach the micro CT resolution, yielding only 2-4 pixels per particle.
- 80µm particles had the smallest standard deviation in particle position with different thresholds, and is the best choice for particle tracking
- Adding 20µm increases hydrogel attenuation and may be useful in future studies to distinguishing the hydrogel from surrounding cartilage
- Strains for unconfined compression match expected values and demonstrate feasibility of hydrogel actuator compression
- Strains for confined compression are lower than expected, which may be due to friction at the confining wall-gel interface
- Radial strains showed weak linear correlations, which may be due to the movement-dependent method of measurement of the gel's center for the reference position. In future studies, a new origin based on a static point in the image will be used to calculate position and displacement radially.
- Other errors as likely due to the skewed nature of the gel and axially uneven bulging during unconfined compression

REFERENCES

[1]Schinagl RM, Gurskis D, Chen AC, Sah RL. J Orthop Res 1997;15:499-506. [2]Palmer AW, Guldberg RE, Levenston ME.Proc Natl Acad Sci U S A 2006;103:19255-60. [3]Spiller KL, Maher SA, Lowman AM. Tissue Engineering;17:281-299. [4]Mow VC, Kuei SC, Lai WM, Armstrong CG. J Biomech Eng 1980;102:73-84.

ACKNOWLEDGMENTS

Supported by a grant from Calit2. Many thanks to Esther Cory and Dr. Robert L Sah.