1 **Title**

2 Ex Vivo Loading of Trussed Implants for Spine Fusion Induces Heterogeneous Strains

3 Consistent with Homeostatic Bone Mechanobiology

4

5 **Article Type**

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7

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Abstract

A truss structure was recently introduced as an interbody fusion cage. As a truss system, some of the connected elements may be in a state of compression and others in tension. This study aimed to quantify both the mean and variance of strut strains in such an implant when loaded in a simulated fusion condition with vertebral body or contoured plastic loading platens *ex vivo*. Cages were each instrumented with 78 fiducial spheres, loaded between platens (vertebral body or contoured plastic), imaged using high resolution micro-CT, and analyzed for deformation and strain of each of the 221 struts. With repeated loading of a cage by vertebral platens, the distribution (variance, indicated by SD) of strut strains widened from 50N control ($4 \pm 114 \mu\epsilon$, mean $\pm$ SD) to 1000N ($-23 \pm 273 \mu\epsilon$) and 2000N ($-48 \pm 414 \mu\epsilon$), and between 1000N and 2000N. With similar loading of multiple cages, the strain distribution at 2000N ($23 \pm 389 \mu\epsilon$) increased from 50N control. With repeated loading by contoured plastic platens, induced strains at 2000N had a distribution similar to that induced by vertebral platens ($84 \pm 426 \mu\epsilon$). In all studies, cages exhibited increases in strut strain amplitude when loaded from 50N to 1000N or 2000N. Correspondingly, at 2000N, 59-64% of struts exhibited strain amplitudes consistent with mechanobiologically-regulated bone homeostasis. At 2000N, vertically-oriented struts exhibited deformation of $-2.87 \pm 2.04 \mu\text{m}$ and strain of $-199 \pm 133 \mu\epsilon$, indicating overall cage compression. Thus, using an *ex vivo* 3-D experimental biomechanical analysis method, a truss implant can have strains induced by physiological loading that are heterogeneous and of amplitudes consistent with mechanobiological bone homeostasis.

1 **Introduction**

2
3 Lumbar spine interbody fusion implants, or cages, are used clinically to induce vertebral fusion
4 as a treatment for degenerative disc disease (Weiner and Fraser, 1998, Zdeblick and Phillips,
5 2003). These cages provide mechanical support between vertebrae and are typically composed of
6 polyether ether ketone (PEEK) or metal, such as titanium. Many cages are designed for usage
7 with biologics (Agarwal et al., 2009, Bishop et al., 1996, Burkus et al., 2002a), such as native or
8 synthetic bone grafts, or growth factors including bone morphogenetic protein (BMP).

9
10 Bone formation and remodeling involve mechanobiology, a complex process by which
11 mechanical loads influence the osteogenic biological response (Cowin and Hegedus, 1976, Frost,
12 2003, Mow and Huiskes, 2004, Oftadeh et al., 2015, Turner, 1998). With controlled loading *in*
13 *vitro* or *in vivo*, strain amplitudes up to $\sim 200\mu\epsilon$ (microstrain, 10^{-6} strain) result in net bone
14 resorption, ~ 200 - $1500\mu\epsilon$ preserve bone homeostasis, and $>1500\mu\epsilon$ promote bone formation
15 (Akhter et al., 1998, Burger et al., 1992, Cullen et al., 2001, Duncan and Turner, 1995). Such
16 mechanobiological strain regimes may be useful to facilitate bone ingrowth into fusion devices
17 (Reid et al., 2011). Cage functionality has been described by effects on range of motion *ex vivo*,
18 while clinical effectiveness is evaluated by patient outcome measures and fusion rate (Burkus et
19 al., 2002b, Fogel et al., 2014). Intrinsic cage mechanical function has been assessed
20 experimentally for overall structural properties and theoretically by finite element modeling
21 (Adam et al., 2003, Bevill and Keaveny, 2009, Choi et al., 2013, Fogel et al., 2014). Local
22 mechanical properties of trabecular bone and other tissues have been quantified *in vitro*
23 (Keaveny et al., 2001, Muller, 2009, Nagaraja et al., 2005, Suffoletto et al., 2006). However,

loading-induced local deformations and strains of spine fusion implants have not previously been quantified.

With the advent of 3-D printing for implants, cages with internal architectures have been introduced. Cages with an open-space trussed architecture may distribute load throughout the cage, allow lateral and axial communication, and provide space for bone growth and fusion (Kiapour et al., 2011). In engineering, truss structures are comprised of linear elements (struts) that are connected at joints (vertices). In response to applied loads, struts are considered to be in either tension (positive) or compression (negative), with a truss in static equilibrium having a sum of forces equal to zero. Increased loads result in higher magnitude strut forces in both tension (more positive) and compression (more negative), but the net force sum remains zero. We expect that for all the struts in a cage, increased applied load will lead to increased variability of strut deformations/strains (force), with little change to the overall deformation and strain mean (~zero), consistent with a truss structure.

Image-based tracking of intrinsic or affixed markers allows experimental estimation of local strains. More traditional experimental methods elucidate overall implant deformation and strain with platen-to-platen displacement transducers or extensometers, or local strains with mounted strain gauges. These approaches would yield only overall deformation and be difficult to affix to individual and internal struts. Imaging with x-ray or fluoroscopy are often employed to determine the position of spine cages (Burkus et al., 2002a, Nayak et al., 2013). Such imaging methods, along with image analyses such as texture analysis, can delineate bone deformation *in vitro* to a resolution of $1.23\mu\text{m}$ and strain of $300\mu\epsilon$ during physiological loading (Bay et al.,

1999a, Bay et al., 1999b). With marker-based tracking, high contrast markers are affixed to a structure and the movement of marker positions is assessed with imaging. Such approaches have used extrinsic markers, such as implanted cardiac beads, and intrinsic markers, as with fluorescence microscopy to determine depth-dependent properties of articular cartilage (Schinagl et al., 1997, Zerhouni et al., 1988). Such image-based methods could be similarly applied to assess the mechanics of structures such as spine cages.

For orthopaedic biomechanics analysis, 3-D imaging modalities such as μ CT, CT, or MRI have begun to be used (Meisel et al., 2008, Williams et al., 2005). Image analysis of fiducial centroid position typically allows localization at sub-voxel resolution due to the contribution of partial-volume effects at the marker surface (Penney et al., 1998, Van Sint Jan et al., 2002). Regional femoral stem micro-motion has been quantified by tracking affixed markers using μ CT (Gortchacow et al., 2011). For 3-D printed truss cages, the thin, roughened titanium material composition facilitates x-ray or (μ)CT analysis of internal cage structure. The overall objective of this study was to quantify the local strut-associated strains of cages loaded between vertebral body or contoured plastic platens *ex vivo* after placement of markers, with a particular emphasis on strain distribution, described by both variance and mean.

Methods

Study Design

For four 4WEB ALIF cages (#1-#4), strut deformation and strain were determined for (1) a single cage (#1) loaded repeatedly with vertebral platens, (2) multiple cages (#2 and #3) loaded with vertebral platens, and (3) a single cage (#4) loaded with contoured plastic platens (**Table 1**).

Study 1: Intra-cage Response to Repeated Loading by Vertebral Platens.

To quantify the distribution of strain between struts, and strain reproducibility, for a given cage loaded with vertebral platens, repeated measures (n=3) of a single cage subjected to 50N, 1000N, and 2000N were made. Strain distribution variance, indicative of increased number of struts in compression (negative) and tension (positive), was compared to 50N control for 1000N and 2000N loads using Levene's tests (median). Comparisons of repeated 50N load vertebral platen control were also made to quantify the sensitivity of strain detection.

Study 2: Inter-Cage Response to Loading by Vertebral Platens.

Subsequently, to quantify the reproducibility of load-induced strain between cages, two other cages were tested individually, at 50N and 2000N by vertebral platens and compared to 50N control. Strain variance was compared to 50N control at 2000N using Levene's test.

Study 3: Intra-Cage Response to Repeated Loading by Plastic Platens.

To determine the extent and reproducibility of load-induced strain response to contoured plastic platens, a single cage was loaded repeatedly (n=2) to 50N and 2000N. Strain variance at 2000N was compared with 50N control and that for study 1, 2000N by Levene's tests.

For all studies, strut deformation, strain amplitude, and corresponding percentage of struts exhibiting strains within the combined mechanobiological homeostasis+formation ($\geq 200\mu\epsilon$) ranges were described statistically for each experimental group, and also compared between groups by 1-way ANOVA with *post-hoc* Tukey tests. Also, mean strain was compared with value 0 between all groups. Strut deformation and strain data are reported as mean $\pm$ SD calculated in two ways, by assessing (1) the distribution for struts within each trial/cage (indicated as trial/cage-averaged) to quantify the distribution of deformation and strain internal to individual cages, or (2) the average of all struts within each trial/cage (indicated as strut-averaged) to quantify the variation between trials/cages. In each study, deformation and strain are reported as the distribution, vertical strut distribution, and amplitudes. All groups were tested for normality by the Kolmogorov-Smirnov test. The significance threshold for all statistics was set to $\alpha=0.05$. Statistics were performed using Excel (v2013, Microsoft, Redmond, WA, USA) and SPSS (v22, IBM, Armonk, NY, USA).

Sample Preparation

Anterior lumbar interbody fusion (ALIF) cage implants and platens (vertebral body or contoured plastic) were prepared for mechanical loading. ALIF spine implants were provided by 4WEB

Medical. The implants had a trussed design (40mm x 27mm x 16mm, LxWxH) with roughened titanium struts (1.25mm diameter). Zirconia spheres (0.5mm diameter) were attached using cyanoacrylate at each vertex (intersection of multiple truss struts) to serve as fiducial markers (66 total) (**Figure 1**). Additional spheres (n=12) were placed at the center of some struts, and used to validate the linearity of displacement and thus consistency of strain along a single strut (data not shown). Human vertebral bodies (L4 & L5) from one cadaveric donor (51 year old female) were obtained from a tissue bank (University of Miami) and stored at -80°C. The vertebral bodies were prepared to simulate a fusion condition by excising the L4-L5 intervertebral disc, cutting with an oscillating saw parallel to the endplates and then a rongeur. Plastic platens comprised of polysulfone (an autoclaveable inert plastic) were designed to mimic the vertebral bodies and contoured to provide a counter-surface match to the cages. The prepared vertebral bodies or contoured plastic served as loading platens, superior and inferior to the cage.

Mechanical Loading

Compressive loads were applied *ex vivo* to cages between loading platens via a custom μ CT-compatible compression device with in-line load monitoring. The stand-alone cage was inserted anteriorly without graft material between the vertebral or plastic platens, and the platen-cage-platen complex was loaded. This loading device was designed to fit within a μ CT scanner bed and apply compressive loads without interfering with the x-ray transmission paths. A swivel plate allowed for 3 rotational degrees of freedom at one vertebral body to allow the natural conformation of the platen to the cage (**Figure 2**). For samples using donor vertebral body platens, the bone-cage-bone complex was sealed in a fluid-tight bag with phosphate-buffered

1 saline within to maintain high humidity. Compressive loads of 50N, 1000N, or 2000N were
2 applied via the platens to the implant, allowing 10 minutes of relaxation to equilibrium, as
3 determined by the in-line load cell (9000N capacity), prior to imaging. Load levels were selected
4 to represent physiological amplitudes (Nachemson, 1975, Schultz et al., 1982). The 50N load
5 was applied to maintain the cage between the loading platens. Between trials, 30 minutes was
6 allowed for relaxation, during which time, the sample and loading apparatus were disassembled
7 and reassembled.

9 *Imaging & Analysis*

11 High resolution 3-D images were acquired for each load level and processed to determine
12 location of fiducials. The loaded implant was imaged on a micro-Computed Tomography
13 scanner (Skyscan 1076, Bruker-microCT, Kontich, Belgium) with $(9\mu\text{m})^3$ voxel size, applying an
14 electrical potential of 100kVp and current of 100uA, using a 0.038mm copper + 0.5mm
15 aluminum filter. A beam-hardening correction algorithm was applied prior to image
16 reconstruction. Images were reconstructed and thresholded (85-255 grayscale values) to segment
17 the radiopaque spheres as fiducials. Image processing included removal of speckles <40000
18 voxels and Gaussian filtering (radius=10, CTAn, Bruker-microCT, Kontich, Belgium) to remove
19 noise and improve fiducial sphericity and reproducibility. Automated individual object 3-D
20 analysis was performed to calculate fiducial 3-D centroids (CTAn).

22 Control studies confirmed that a sub-voxel resolution of $\sim 1\mu\text{m}$ was achieved for detecting load-
23 induced bead displacement. A spring was fitted with radiopaque beads, similar to the cage, with

five beads spaced regularly across the length of the spring (38mm), and subjected to 0, 10 μ m, and 20 μ m of overall compression. After each compression, the sample was imaged by μ CT at (9 μ m)³ voxel resolution, and bead centroids determined by image processing as described above. For central (N=3) spheres, deviation of the spheres from the predicted position in the x, y, and z image axes were 0.6 $\pm$ 0.4 μ m, 0.5 $\pm$ 0.3 μ m, and 1.0 $\pm$ 0.5 μ m, respectively, averaged over two trials (mean $\pm$ SD). The variability in each measure represents $\sim$ 1/10 voxel resolution for centroid determination, which is expected due to partial volume effects (Verhulp et al., 2004). Thus for the average strut length of $\sim$ 10mm, the z-directed strain resolution was 1 μ m/10mm=100 $\mu\epsilon$.

Data Reduction/Calculations

Deformation, engineering strain, and deformation and strain amplitudes were calculated for each truss strut using pairs of fiducials in an automated fashion using software implemented in MATLAB 2012b (MathWorks, Natick, MA, USA). Fiducial centroids were registered between pairs of scans via 3-D linear transformation (translation and rotation). Using 3-D centroids, struts were automatically defined as pairwise combinations of fiducials, which matched the 221 struts on each cage. A portion of the anterior face of the cage had an instrumentation attachment plate, and connected struts (n=12) were not analyzed. In each of study 1 and the vertebral platen control study, two peripheral fiducials were observed on μ CT to make direct contact with the loading platen; thus, the 15 associated struts were excluded from analysis. Strut lengths were calculated for each strut in each trial as the scalar linear distances between sphere pairs in the 50N load conditions (9.0 $\pm$ 2.2mm, N=221). Deformation was calculated between pairs of scans as the scalar change in length of each strut from 50N initial load to 50N, 1000N, or 2000N final

load. Engineering strain (negative in compression, positive in tension) was calculated for each strut as the deformation divided by the initial strut length at 50N. Deformation and strain amplitudes were taken as the absolute values (magnitude) of deformation and strain, respectively. Vertical cage deformation and strain were calculated by averaging values for central axially-oriented struts (N=10).

Results

In all studies (described in detail below), cages subjected to 1000N or 2000N load exhibited strut strain distribution variances and amplitudes higher than those of 50N controls. Strain distribution variance for 1000N and 2000N, and amplitude for 2000N, was greater than that of 50N controls in all studies, and those for 2000N were greater than for 1000N in study 1 ($p<0.05$, all pairs). Correspondingly, percentages of strut strain amplitudes in the combined homeostasis+formation ($\geq 200\mu\epsilon$) ranges for 2000N loads were greater than those for 50N controls ($p<0.05$, all pairs). Normality testing on strut-averaged strain showed normally distributed strains for 50N vertebral ($p=0.20$) and plastic platen ($p=0.20$) controls, and 2000N groups for studies 1 ($p=0.20$), 2 ($p=0.06$), and 3 ($p=0.20$). Study 1, 1000N and study 3, 2000N trended towards normality ($p<0.05$). Thus, application of 2000N by either vertebral or plastic platens caused strain distribution to broaden and strain amplitude to increase compared to 50N controls.

Study 1: Intra-cage Response to Repeated Loading by Vertebral Platens.

Strain Distribution

1
2 In repeated measures of cage #1, physiological loads of 1000N and 2000N, applied with
3 vertebral platens, caused a distribution of strut strains with both positive (tension) and negative
4 (compression) values (**Table 2, Figure 3**). Trial-averaged (n=3) strain of all struts showed
5 distribution (variance, indicated by SD) that widened from 50N control ($4\pm114\mu\epsilon$) to 1000N ($-$
6 $23\pm273\mu\epsilon$) and 2000N ($-48\pm414\mu\epsilon$), and between 1000N and 2000N (**Table 2**, $p<0.05$, all pairs).
7 Although strain variance increased (larger positive values and more negative values about the
8 mean), the means of the strain distributions were not different from zero for both 1000N
9 ($p=0.99$) and 2000N ($p=0.94$). Vertical (central axially-oriented struts, N=10) strut-averaged
10 deformation was $0.17\pm0.60\mu\text{m}$ and $-1.87\pm0.82\mu\text{m}$ for 1000N and 2000N load, respectively, and
11 the corresponding vertical strain was $12\pm39\mu\epsilon$ and $-124\pm52\mu\epsilon$ (**Table 3**).
12

13 *Strain Amplitude and Mechanobiological Ranges*

14

15 Strut-averaged strain amplitude also increased with load, from $193\pm33\mu\epsilon$ at 50N to $355\pm35\mu\epsilon$ at
16 2000N ($p<0.05$), with that from 50N to 1000N, $251\pm10\mu\epsilon$, not reaching significance ($p=0.306$,
17 **Table A.1**).
18

19 These strain amplitudes corresponded to 49% and 64% homeostasis ($200\text{-}1500\mu\epsilon$) and 0% and
20 1% formation ($>1500\mu\epsilon$) for 1000N and 2000N load, respectively. Percentages of strut strain
21 amplitudes in the combined homeostasis+formation ($\geq 200\mu\epsilon$) ranges for 2000N were greater
22 than those for 50N ($p<0.05$), but not significantly for 1000N ($p=0.08$).
23

Vertebral Platen Control

Under repeated 50N tare load, comparison of image datasets yielded strut strain indicative of noise, i.e. relatively small and symmetrically distributed. Repeated trial-averaged ($n=2$) deformation and strain distribution was normally distributed ($p=0.20$, **Figure 4A,C**) with small strut-averaged strain amplitudes of $193\pm 33\mu\epsilon$, respectively (**Table 2**), consistent with control loading study, considering the z-direction displacement resolution ($\sim 100\mu\epsilon$) of two beads. Strain amplitudes corresponded to 37% homeostasis and 0% formation. This baseline measurement for noise indicates that deformation and strain could be assessed with high sensitivity.

Study 2: Inter-Cage Response to Loading by Vertebral Platens.

Strain Distribution

The strain distribution at 2000N was similar for two additional cages (#2 and #3, **Table 2**, **Figure 5**). Relative to strain distribution at 50N, strain at 2000N, $23\pm 389\mu\epsilon$, increased in variance ($p<0.05$) but not in mean ($p=0.99$). Vertical strut-averaged deformation and strain distribution was $-2.87\pm 2.04\mu\text{m}$ and $-199\pm 133\mu\epsilon$, respectively (**Table 3**).

Strain Amplitude and Mechanobiological Ranges

Corresponding strut-averaged strain amplitude also increased consistently between cages, from $193\pm 33\mu\epsilon$ at 50N to $337\pm 48\mu\epsilon$ at 2000N ($p<0.05$, **Table A.1**). These strain amplitudes

1 corresponded to 59% homeostasis and 1% formation. Percentage of strut strain amplitudes in the
2 combined homeostasis+formation ranges was greater for 2000N than 50N control ($p<0.05$).

4 ***Study 3: Intra-Cage Response to Repeated Loading by Plastic Platens.***

6 *Strain Distribution*

8 Repeated loading of cage #4 at 2000N with plastic platens induced strains with a distribution
9 similar to those induced by vertebral body platens (**Table 2, Figure 6**). Trial-averaged ($n=2$)
10 strain of all struts showed widened distribution (increased variance), from 50N control
11 ($38\pm127\mu\epsilon$) to 2000N ($84\pm426\mu\epsilon$, $p<0.05$). Strain variance was similar to that of study 1 for
12 2000N ($p=0.92$). Also similar to study 1, the means of the strain distributions were not different
13 from zero at 2000N ($p=0.71$). Vertical strut-averaged deformation and strain distribution was -
14 $3.30\pm0.64\mu\text{m}$ and $-224\pm41\mu\epsilon$, respectively (**Table 3**). Using the vertical strut deformations of the
15 loaded cage (study 3), the apparent stiffness and effective modulus of the cage under axial
16 compression were $618,000\pm118,000\text{N/mm}$ and $12.4\pm2.4\text{MPa}$, respectively.

18 *Strain Amplitude and Mechanobiological Ranges*

20 Strut-averaged strain amplitude also increased with load from 50N control ($193\pm33\mu\epsilon$) to 2000N
21 ($336\pm1\mu\epsilon$, $p<0.05$, **Table A.1**). Compared with donor vertebral body platens (study 2, 2000N),
22 strut-averaged strain amplitudes were not significantly different ($p=0.97$). Strain amplitudes
23 corresponded to 59% homeostasis and 0% formation for 2000N load. Percentages of strut strain

1 amplitudes in the combined homeostasis+formation ranges for 2000N were greater than those for
2 50N control ($p<0.05$). Thus, loading with plastic platens produced strains similar to those
3 induced by loading with vertebral bodies as platens.

4 5 *Plastic Platen Control*

6
7 For repeated 50N plastic platen control, trial-averaged ($n=2$) strain distribution was normally
8 distributed ($p=0.20$, **Figure 4B,D**) with strut-averaged strain amplitudes of $132\pm14\mu\epsilon$,
9 respectively, similar to vertebral platens ($p=0.33$, **Table A.1**). Corresponding percentages of
10 strain amplitudes in mechanobiological ranges were 21% homeostasis and 0% formation.

11 12 **Discussion**

13
14 In the present study, local deformations and strains were quantified for *ex vivo* loaded trussed
15 ALIF cages using μ CT to track affixed fiducials. Struts exhibited a strain distribution with
16 approximately equal numbers of struts in compression as tension and centered about zero.
17 Vertical loaded strains, as determined by central axially-oriented struts, showed mainly
18 compressive responses. Repeated compression of a single cage between vertebral platens
19 exhibited deformation and strain amplitudes that increased with physiological load. Loading of
20 multiple cages with vertebral platens indicated comparable strain responses, as did repeated
21 loading with contoured plastic platens.

1 The study design and approach involved a number of tradeoffs. Cage loading was axial
2 compression, simulating the primary loading applied to the spine; more complex loading
3 conditions, such as bending, torsion, and shear, were not analyzed. Static loading was selected
4 rather than dynamic loading to facilitate imaging and analysis. Strain was calculated as
5 engineering strain from end-to-end strut deformation and length, and assumed to be represent
6 simple compression/tension. Shear, torsion, and bending strains were not assessed.

7
8 There was a substantial variation in the deformation of individual struts between trials, although
9 the overall distribution of strut deformation and strain was consistent, with roughly equal number
10 of struts in compression and tension within each trial. Variation between trials for individual
11 struts is likely due to the sensitivity of local strut mechanics to the end-loading conditions of the
12 platens, similar to that recognized for biomechanical testing of trabecular bone (Keaveny et al.,
13 1997). The strain distribution was consistent with a truss in static equilibrium, which has net zero
14 load (some negative, some positive) within the structure. Non-linear overall load-
15 displacement/strain relationships are likely due to complex loading condition between the cage
16 and platens. The results of the present study extend previous experimental mechanical analysis of
17 interbody fusion cages that address the overall behavior of the vertebral-cage complex or large
18 spine segments (Coe et al., 1989, Nayak et al., 2013, Rapoff et al., 1997, Wang et al., 2014).

19
20 The amplitudes of cage strut strains, determined in the present study, are in the range of those
21 mediating mechanobiological homeostasis of bone. Loading up to 1500 $\mu\epsilon$ induced a five-fold
22 increase in bone formation in rat tibiae (Cullen et al., 2001). Induced fluid flow may transduce
23 and amplify the solid strain to higher effective mechanoregulatory signals in the implant (Zhao et

1 al., 2015). In the post-implant fusion situation, such strain and mechanobiology may be highly
2 relevant to the osteogenic biological response. Bone ingrowth through the cage would likely alter
3 the mechanical environment over time, sharing load transmission with the cage.

4
5 The 3-D printed nature of the trussed cages facilitates their design for operation in a targeted
6 mechanobiological response regime. While the present study analyzed and quantified
7 macroscopic strut deformation and strain, microstructural factors may also affect implant
8 performance. Surface roughness, asperities, texture, and charge may modulate local interactions
9 with cells, and subsequent responses, including formation of oriented trabeculae. The similar
10 response of vertebral and contoured plastic platens facilitates future *in vitro* studies of cage
11 mechanobiology, with the latter eliminating the complexity of effects on cage-attached cells of
12 components in post-mortem bone.

13
14 In conclusion, the present study provides the first quantification of localized strains throughout a
15 cage structure when subjected to physiological loads. Loaded strut strain amplitudes were largely
16 consistent with mechanobiological homeostasis of bone, which may be an important property for
17 interbody fusion. Further studies are needed to investigate modulation of strain, effective
18 modulus, and mechanobiological cellular response through cage design.

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Figure Legends

Fig. 1. Structure of 4WEB ALIF cage with affixed fiducial sphere markers. 3-D microCT volume model of implant (gray) with fiducials attached (red) in (A) posterior, (B) right anterior oblique, and (C) left anterior oblique views. (D) Posterior view photo. Right and Left sides are indicated by “R” and “L”, respectively. Red dots in (A-C) represent attached fiducials.

Fig. 2. Cage loading configuration. (A) Schematic. Photos with (B) vertebral body and (C) plastic platens. Swivel plate allows superior platen to conform to implant lordosis. Arrow indicates direction of force [F] application. Red dots in (A) represent attached fiducials.

Fig. 3. Intra-cage strain distribution for repeated loading by vertebral platens (study 1). Histograms of strain for all struts in repeated (A) 1000N and (B) 2000N measures. Representative trial strain colormaps for (C) 1000N and (D) 2000N shown on cage layout. Error bars represent SD (n=3). Positive strain values indicate tension and negative strain values indicate compression.

Fig. 4. Intra-cage strain distribution for controls. Histogram of strain for all struts for (A) vertebral platen and (B) plastic platen controls. Representative trial strain colormaps shown on cage layout for (C) vertebral platen and (D) plastic platen controls. Error bars represent SD (n=2). Positive strain values indicate tension and negative strain values indicate compression.

1 **Fig. 5. Inter-cage strain distribution for loading by vertebral platens (study 2).** (A)

2 Histogram of strain for all struts in multiple cage 2000N measures. (B) Representative cage
3 strain colormap shown on cage layout. Error bars represent SD (N=2). Positive strain values
4 indicate tension and negative strain values indicate compression.

6 **Fig. 6. Intra-cage strain distribution for repeated loading by plastic platens (study 3).** (A)

7 Histogram of strain for all struts in repeated 2000N measures. (B) Representative trial strain
8 colormap shown on cage layout. Error bars represent SD (n=2). Positive strain values indicate
9 tension and negative strain values indicate compression.

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1 **Table 1.** Study groups for each aim for unloaded and loaded cages with single-cage repeated
2 trials (n) or multiple cages (N).

Study	Platens	Load [N]	n	N
1	vertebral	50	3	1
		1000	3	1
		2000	3	1
2	vertebral	50	1	2
		2000	1	2
3	plastic	50	2	1
		2000	2	1

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- 1 **Table 2. Deformation and strain of all struts.** For deformation (ΔL) and strain (ϵ) of struts in
- 2 each control and loaded group, mean, SDs (variation between trials/cages) of averaged strut
- 3 values, and SDs (variation between the struts within trial/cage) of average trial/cage values.

Study	Platens	Load [N]	Mean		SD, Strut			SD, Trial/Cage		
			ΔL [μm]	ϵ [$\mu\epsilon$]	ΔL [μm]	ϵ [$\mu\epsilon$]	n	ΔL [μm]	ϵ [$\mu\epsilon$]	n
control	vertebral	50	0.04	4	0.96	99	2	0.95	114	206
1	vertebral	1000	-0.19	-23	0.33	35	3	2.29	273	206
1	vertebral	2000	-0.40	-48	0.17	27	3	3.48	414	206
2	vertebral	2000	0.33	23	0.93	107	2	3.45	389	221
control	plastic	50	0.34	38	0.21	25	2	1.12	127	221
3	plastic	2000	0.79	84	0.03	2	2	3.59	426	221

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- 1 **Table 3. Deformation and strain of vertical struts.** For deformation (ΔL) and strain (ϵ) of
- 2 vertical struts (N=10), mean, SDs of averaged strut values, and SDs of average trial/cage values.

Study	Platens	Load [N]	Mean		SD, Strut			SD, Trial/Cage		
			ΔL [μm]	ϵ [$\mu\epsilon$]	ΔL [μm]	ϵ [$\mu\epsilon$]	n	ΔL [μm]	ϵ [$\mu\epsilon$]	n
control	vertebral	50	-0.62	-41	0.19	14	2	0.75	49	206
1	vertebral	1000	0.17	12	0.60	39	3	2.09	148	206
1	vertebral	2000	-1.87	-124	0.82	52	3	3.51	238	206
2	vertebral	2000	-2.87	-199	2.04	133	2	3.80	264	221
control	plastic	50	1.00	66	0.32	20	2	0.44	30	221
3	plastic	2000	-3.30	-224	0.64	41	2	4.30	295	221

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