Design and analysis of a novel mechanical loading machine for dynamic *in vivo* axial loading

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This paper describes the construction of a loading machine for performing *in vivo*, dynamic mechanical loading of the rodent forearm. The loading machine utilizes a unique type of electromagnetic actuator with no mechanically resistive components (servotube), allowing highly accurate loads to be created. A regression analysis of the force created by the actuator with respect to the input voltage demonstrates high linear correlation ($R^2 = 1$). When the linear correlation is used to create dynamic loading waveforms in the frequency (0.5–10 Hz) and load (1–50 N) range used for *in vivo* loading, less than 1% normalized root mean square error (NRMSE) is computed. Larger NRMSE is found at increased frequencies, with 5%–8% occurring at 40 Hz, and reasons are discussed. Amplifiers (strain gauge, linear voltage displacement transducer (LVDT), and load cell) are constructed, calibrated, and integrated, to allow well-resolved dynamic measurements to be recorded at each program cycle. Each of the amplifiers uses an active filter with cutoff frequency at the maximum *in vivo* loading frequencies (50 Hz) so that electronic noise generated by the servo drive and actuator are reduced. The LVDT and load cell amplifiers allow evaluation of stress-strain relationships to determine if *in vivo* bone damage is occurring. The strain gauge amplifier allows dynamic force to strain calibrations to occur for animals of different sex, age, and strain. Unique features are integrated into the loading system, including a weightless mode, which allows the limbs of anesthetized animals to be quickly positioned and removed. Although the device is constructed for *in vivo* axial bone loading, it can be used within constraints, as a general measurement instrument in a laboratory setting. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.3687781>]

I. INTRODUCTION

Investigators evaluating bone growth utilize loading machines to induce physiological effects within an *in vivo* animal model. Building the loading machine is something that bone researchers have done to save money and to introduce custom requirements.^{1–3} Several investigators have reported using different types of technologies (i.e., piezoelectric, stepper, and pneumatic) for custom made loading machines for *ex vivo* and *in vivo* research,^{4–15} although how the devices are made is not described, nor how well they work quantified.

There are many constraints unique to a dynamic loading system used on live animals. First, because the specimens are generally under short acting anesthetic, researchers have to be able to put the animals into the loading apparatus and as well as remove them quickly. Second, the loading machine needs to remain stable during any motion created by anesthetized animals, and thus, it is desirable to bypass the use of a proportional-integrative-derivate (PID) controller. Third, the loading system should be able to create and record dynamic loads in the frequency range of 0.5–10 Hz most often evaluated,^{6,7,13,16–18} as well as determine if damage (stress-strain analysis) is occurring. Fourth, the system should be able to do force to strain calibration within specific areas of the bone being evaluated.^{3,5,6,16–18} Finally, the system has to

be easy to create, and ideally this means using linear components in a non-real-time setting.³ This paper documents the creation of an *in vivo* dynamic loading system, which is based off a unique frictionless electromagnetic actuator. The accuracy of this loading system is quantified at different load and frequencies used for *in vivo* loading. In addition, unique methods which allow quick positioning of animals in the loading machine are detailed.

II. DESIGN

A. The actuator and servo drive

An electromagnetic actuator typically uses bearings, bushings, and grease to reduce friction from the iron core moving through the coils. Since most applications of an actuator are done in position control, which is monitored by sensors, the resistance of these components has no effect. Yet, when running under the load control, these components reduce the accuracy of the applied load. Thus, an ideal electromagnetic actuator will not have bushings and bearings, nor grease (the latter is also not clean in a laboratory environment). To distinguish this unique type of electromagnetic actuator from others, it will be referred to as a frictionless electromagnetic linear actuator (FELA). The FELA utilized is a STB1116 ServoTube (Copley Controls, Canton, MA, USA) and is powered by an Accelnet Servodrive (Copley Controls, Canton, MA, USA). Copley Controls is currently the only company that manufactures this type of frictionless electromagnetic drive.

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FIG. 1. (Color online) Linear regression ($n = 3$) is performed to access the linearity of the FELA. Since the system is highly linear, the equation can be utilized to create computer controlled dynamic force waveforms.

The force produced by the FELA in load control (current mode) has a linear response to an analog voltage input on the servo drive (Fig. 1). The linear relationship was determined by varying an analog voltage from a computer data acquisition (DAQ) card to the analog input of the servo drive and recording the load it produces. Linear regression then determines the equation between the force and voltage as well as checks for correlations. Note if the correlation coefficient of the regression is not 0.99 or higher, there is some type of resistance keeping the device from working in a linear manner.

B. Mechanical design considerations to improve resolution

The FELA is mounted with the forcing unit (which houses the coils) fixed to a solid support, and only the thrust rod is permitted to move (Fig. 2). Since the thrust rod weighs five times less than the forcing unit, this mounting arrangement reduces the work the FELA has to do to oppose gravity. For optimal operation, the forcing unit and thrust rod would be operated horizontally rather than in a vertical arrangement, but since gravity assists in holding the mice limbs in place during loading, the vertical arrangement was used.

C. Positional data: The linear voltage displacement transducer

A model 240 DC LVDT (Transtek, Ellington, CT, USA) with 102 mm stroke range was utilized to record micrometer displacements. The dc linear voltage displacement transducer (LVDT) outputs an analog voltage which is linear over its 102 mm stroke range, and has $\sim 5 \mu\text{m}$ resolution after analog to digital (A/D) conversion. In order to improve displacement measurements of the LVDT, a linear amplifier was created (Fig. 3). The amplifier improves resolution at the expense of having a shorter working range (due to saturation). The LVDT amplifier is an inverting operational amplifier (op-amp) with

the input resistor (R_i) set to $2\,200 \Omega$ to reduce electrical loading of the LVDT. The gain produced by the op-amp is then adjusted by changing the value of the feedback resistor (R_f),

$$\text{Gain} = \frac{-R_f}{R_i}. \quad (1)$$

By utilizing a series of dual inline package (DIP) switches, a set of different feedback resistors (R_f) could be

FIG. 2. The mechanical arrangement of the bone loading device has all components in series. In this vertical arrangement, the forcing unit will have to offset the effects of gravity on the thrust rod, but this is a compromise as it is easier to position specimens.

FIG. 3. An amplifier for the dc LVDT was created so that high displacement resolution could be obtained. The use of DIP switches (only two shown, SW = 8, SW = 6) allows a trade off in amplifier gain and working range to be made with different size animal models.

used to create a user changeable gain (Table I). Thus, the amplifier served the purpose that many different length dc LVDT units could do, but without having to remount the units during experimentation.

By adding a capacitor to the feedback path of the op-amp, a low-pass active filter is configured in which the signal-to-noise ratio (SNR) is improved. The filtering capacitor (C) was determined using a RC time constant that produces a 50 Hz cutoff frequency (f_c). Since there are different R_f , a unique C is found for each in order to keep f_c constant.

The linear relationship between the displacement of the LVDT and sampled voltage from the output of LVDT amplifier (with each R_f) was determined by displacing the core known distances via an array of feeler gauges and then performing linear regression (Table I). This equation governs the

relationship through two linear devices (LVDT and LVDT amplifier).

The linear equation governing the LVDT was found to be useful for determining other parameters than just displacement. The displacement resolution (Table I), defined as the smallest peak to peak displacement which can be distinguished, is inferred from the slope. Since the LVDT has infinite resolution, the resolution limit is set by the minimum voltage that can be resolved after sampling, and in this case is ~ 1 mV,

$$\text{LVDT Resolution } (\mu\text{m}) = \frac{0.001 \text{ V}}{\text{slope}} \times 1000. \quad (2)$$

The slope also allows us to determine the working range (area inside of saturation) of the amplifier for each R_f (Table I). This range was found to be proportional to the sum of the op-amp supply voltages divided by the linear slope,

$$\text{LVDT working range} = \frac{V_{cc} + |-V_{cc}|}{\text{slope}} \times 1000. \quad (3)$$

Note that this equation demonstrates that increasing the supply voltages of the op-amp allows a larger working range.

D. Load cell and strain gauge amplifier

The signals from both a strain gauge and load cell are amplified and filtered around the frequencies used for *in vivo* loading. The first stage of this circuit is a differential amplifier which amplifies the difference between each side of the

TABLE I. The gain, working range, and filter values of the adjustable dc LVDT amplifier.

Gain	R_f (k Ω)	C (μ H)	Slope (V/mm)	Resolution (μ m)	Range (mm)
1	2.2	1.5	0.1	9.79	101.60
2	4.7	0.68	0.22	4.63	92.68
4.5	10	0.33	0.43	2.33	46.60
10	22	0.15	1.06	0.94	18.90
15	33	0.10	1.56	0.64	12.79
21	47	0.068	2.6	0.39	7.70
31	69	0.047	5.21	0.19	3.84
45	100	0.033	11.91	0.08	1.68

FIG. 4. A differential amplifier (INA121) is used to take the difference from each side of the Wheatstone bridge so that the signal from the load cell and strain gauge can be amplified. A series of switches (only two SW shown) allow the gain of the op-amp (LM741) to be adjusted, giving a trade off in load resolution to the maximum load which can be measured (saturation). A trimpot is used to adjust the dc level out of the instrumentation amplifier.

Wheatstone bridge (without removing the dc voltage, the amplifier will saturate). After the differential is taken, the signal is amplified with an op-amp (Fig. 4), yielding a total gain which is the product of that from the op-amp (Eq. (1)) and instrumentation amplifier (INA 121 datasheet):

$$\text{Gain} = -\left(1 + \frac{50 \text{ k}\Omega}{R_g}\right) \frac{R_f}{R_i}. \quad (4)$$

As with the LVDT amplifier, a series of dip switches can be implemented to vary the gain while keeping f_c constant.

The load cell and its amplifier can be calibrated in the same manner as the LVDT. Performing regression on both devices is possible since each is linear. This regression can be performed by placing known weights onto the load cell to determine the output voltage from the amplifier.³ After the linear equation is found, it is applied to the amplified voltage so it is converted to load.

In order to measure strain, another load cell amplifier was built and connected to a Wheatstone bridge containing a strain gauge in quarter bridge configuration (Fig. 4). Shunt calibration was performed by placing a known resistance in parallel with the strain gauge in order to simulate strain. The calculation of the shunt resistance (R_c) is performed (from Vishay technical note TN-514) with R_G the resistance of the gauge, ϵ_s the simulated strain, and F_G the gauge factor provided by the strain gauge manufacturer:

$$R_c = \frac{R_G \times 10^6}{F_G \epsilon_s} - R_G. \quad (5)$$

After the shunt voltage is read, it is scaled, first with an additive term (until zeroed), and then with multiplicative scalar until the simulated strain is reached.

The strain gauges (Vishay EA-06-015DJ-120) used for recording from mice limbs are small,^{5,6,16–18} and temperature

variation has to be controlled. Due to the small size of the resistive pattern, there is little area to dissipate heat nor is it capable of coupling well into bone (Vishay Technical Note 502). Before taking any voltage readings, the strain gauge is given a few minutes to temperature stabilize. Also, the voltage drop across the strain gauge should be limited to $\sim 0.1\text{--}0.5$ V (Vishay Technical Note 502) to reduce Joule heating. In addition, using the peak to peak strain during dynamic loading limits the effects to long-term temperature variation. Regardless of precaution, the temperature variation of a small strain gauge limits the strain resolution. Attachment of the strain gauge is presented in the Appendix.

E. Maximizing linear relationships to improve resolution

Mapping the linear relationships of the servo drive and amplifiers (strain gauge, LVDT, and load cell) around *in vivo* loading parameters, is the key to improve functionality of the loading machine. With the servo drive, the analog input scaling factor (RAM register $r0 \times 19$) is scalable, and setting it to a value in which the entire 10 V input range is used to produce the maximum experimental *in vivo* load,⁷ maximizes the force resolution (minimum force which can be produced and distinguished). With the load cell/strain gauge amplifier, increasing the gain will reduce the maximum range which can be amplified (saturation), but increases the load resolution (minimum load which can be distinguished with the load cell). This same concept is also used with the LVDT amplifier in which different gain resistors are used to improve the displacement resolution (minimum distance which can be resolved) around the null position, while reducing the working range (Table I). Note that the slope of the linear relationships will change any time the scaling factors are modified, however, they can be precomputed so they are known ahead of time.

FIG. 5. The NRMSE is computed between the measured value from the load cell and the loading waveform (*Sin*), as demonstrated in a 1 Hz, 10 N dataset. The *Sin* only diverges enough from the measured values so that it is visible in the very top and bottom of the waveform. In those areas where the two diverge, the NRMSE increases the most.

F. Computer control

LabVIEW (National Instruments, Austin, Texas) was used to create the dynamic loading waveform from user specified parameters (frequency and load). A function called “simulate signal,” was used to create a single point of a dynamic loading signal during each program cycle (3000 Hz). By applying the linear voltage to force relationship of servo drive, a user specified load would be scaled to the proper voltage for the DAQ output (dynamic waveform). The simulate signal function also allows a static dc offset to be added into the waveform, which was set to ~ 0.3 N to keep the limbs in place. The phase of this loading waveform was made so that it would always start loading with minimum load (0.3 N) and build. The point by point appropriately scaled, simulated signal was sent to an analog output on a PCI 6014 (National Instruments) where it was converted to an analog signal with 16 bits of resolution.

LabVIEW would log the sensor data from each experiment. During each program iteration, the DAQ card would sample the voltage from each sensor (load cell, strain gauge, and LVDT) on an analog input channel with 16 bits of resolution (PCI 6014). After sampling, the linear relationship between the LVDT, strain gauge, and load cell was used to convert the voltage to distance, strain, and force, respectively. Each of the three input channels were plotted to screen and logged to disk while the experiment was running.

G. Weightless mode

The ability to quickly position rodent limbs in the loading system was a large need behind the development of this particular loading machine. It was found that if the rodent lay on its back, the compression cup could be quickly lowered onto a limb and then held in place with a very small constant

force (0.3 N), until the loading cycle could begin. This quick positioning of rodent limbs in the device was accomplished via “weightless mode,” which is a constant voltage supplied to the analog input channel of the servo drive. This constant voltage served to offset the weight of the thrust rod, thus giving the mode its name. Weightless mode is automatically applied both before and after each loading cycle to facilitate the speed in which the specimen could be placed/removed from the loading equipment.

H. Compressional cups

In order to hold animal specimens in place during loading, compressional cups have been utilized by other investigators. The design of the compressional cups can vary with the type of loading being performed. In the case of axial loading of the forearm, the design of compressional cups has been presented by others.^{5,16,17,19}

III. RESULTS

A. Loading accuracy

The accuracy of the loading machine was accessed with the root mean square error (RMSE) between each measured load and the expected. Normalizing the RMSE (NRMSE) with respect to each different load allows it to be expressed as a percentage of error. A comparison of the measured value and simulated *sin* wave is shown in Fig. 5. The NRMSE, increases the most in areas of mismatch between the measured signal and the *sin* wave. When this NRMSE is computed at different frequencies and loads (Table II), the accuracy of the system can be accessed. In the case of frequencies, 0–10 Hz, which have been used for dynamic loading of

TABLE II. The NRMSE, expressed as percent error, determined at different loads and frequencies ($n = 3$, 30 000 samples each).

F (Hz)	1N	5N	10N	15N	30N	50N
0	1.28 ± 0.30	1.2 ± 0.72	0.76 ± 0.49	0.49 ± 0.33	0.44 ± 0.30	0.38 ± 0.30
0.1	0.98 ± 0.01	0.64 ± 0.02	0.39 ± 0.04	0.35 ± 0.07	0.31 ± 0.05	0.28 ± 0.04
0.5	0.97 ± 0.01	0.66 ± 0.05	0.38 ± 0.06	0.41 ± 0.03	0.39 ± 0.03	0.33 ± 0.03
1	0.95 ± 0.05	0.63 ± 0.12	0.36 ± 0.02	0.37 ± 0.05	0.34 ± 0.04	0.29 ± 0.02
5	1.19 ± 0.21	0.71 ± 0.23	0.84 ± 0.15	0.83 ± 0.02	0.81 ± 0.02	0.76 ± 0.02
10	2.52 ± 0.35	1.65 ± 0.34	1.45 ± 0.19	1.58 ± 0.15	1.44 ± 0.10	1.36 ± 0.09
20	3.68 ± 0.30	3.27 ± 0.05	4.34 ± 1.31	3.74 ± 0.20	3.62 ± 0.24	3.57 ± 0.18
40	5.32 ± 0.73	5.44 ± 0.44	6.94 ± 2.41	7.92 ± 1.64	8.04 ± 1.50	8.34 ± 1.40

FIG. 6. (Color online) Cyclic loading to failure of a one-month old mouse forelimb at 2.5 N demonstrates the ability of the LVDT amplifier and load cell to detect damage (change in elastic modulus and creep damage). Only the last ten loading cycles are shown.

rodents,^{6,7,13,16–18} there is an average of less than 1% NRMSE error over the range of loads evaluated.

There are two general trends that can be determined from Table II. The first, there is less accuracy at lower loads (i.e., 1 N), which is expected since motion occurs while the thrust rod is levitated. The second trend is that error increases with frequency and 5%–8% error is seen at 40 Hz.

B. Cyclic loading of the rodent forearm to failure

Cyclic loading to failure was performed on the forearm of one-month old male mouse in order to validate the ability of the loading system to produce and record high-resolution cyclic loads. Although the system will perform cyclic loading *in vivo* (not cyclic loading to failure), these trial runs were performed in order to demonstrate the system is able to record strain in the micrometer range. Sinusoidal cyclic loads (2.5 N, 2 Hz) were applied on these surplus one-month old mice (mice which had been sacrificed in a different experiment) and load and displacement were recorded until failure. A plot of the displacement vs. load shows that as damage occurs (Fig. 6), the displacement becomes larger, causing the loop to become increasingly nonlinear and the slope to change. The loading cycle also demonstrates a small amount

FIG. 7. (Color online) A calibration of strain in response to applied force. This type of calibration will be used for future work with the loading device. It allows different *in vivo* strains to be applied that are within a range known to induce biological effects. This calibration is the average of three different mouse forearms (three-month old female TOPGAL mice) which are each loaded at four different frequencies (0.5, 1, 2, and 4 Hz) and averaged at six load points.

FIG. 8. (Color online) In order to bond with a small mouse bone, the strain gauge (left) is trimmed into its bonding area (right), which reduces its area from 18 mm² to ~ 1.5 mm². A hemocytometer with 1 mm grid spacing (0.25 mm minor tick marks), is used as the background on both images.

of shifting along the displacement axis which is a result of creep (of the soft and hard tissue).

C. Strain calibration in the mouse forearm

A strain gauge was bonded with a mouse leg in order to calibrate strain in response to force.^{5,6,16–18} The strain determined while performing dynamic loading with four different frequencies (0.5, 1, 2, and 4 Hz) shows a linear relationship (Fig. 7). From the regression, the amount of force to apply to achieve the desired microstrain can be determined with confidence ($p = 0.9963$).

IV. DISCUSSION

The dynamic loading machine is able to perform loading with less than 1% NRMSE error within the range of frequencies used for *in vivo* loading (1–10 Hz). The small amounts of error are due to the linearity (Fig. 1) of the force produced by the FELA in response to the computer controlled analog voltage. The small amounts of error in the NRMSE are also due to a load cell amplifier which is able to perform high amplification and active filtering with a cutoff frequency slightly above the loading frequency.

The cyclic loading of a surplus mouse long bone was used to demonstrate the ability to detect damage during *in vivo* dynamic loading. To make these stress-displacement curves possible, highly resolved loading profiles are being generated by the servo drive and the subsequent displacement (Table I) and load cell recordings are accurate. The plotting of the stress-strain curve (Fig. 6) demonstrates that fatigue damage (lengthening of the stress-strain relationship) as well as creep damage (gradual change in length) is occurring. The ability to see damage is critical during *in vivo* cyclic loading to ensure the animals are not being damaged or hurt. The ability to record this damage is dependent on creating a LVDT amplifier which allows displacements to be recorded down to 80 nm.

A strain gauge amplifier is integrated with the loading machine, allowing dynamic strain measurements to be recorded at each program cycle. By allowing temperature stabilization of the strain gauge, and reading the peak to peak strain waveforms, a linear calibration (Fig. 7) produces a R^2 value with confidence. This strain calibration is consistent with others whom have done dynamic loading in mice limbs.^{5,6,16–18} This calibration, in the case of future experi-

ments, allows the force to be determined which will induce a strain within a specific regions of the bone. This calibration will be repeated in each animal with different age, sex, and strain in which a biological response will be evaluated.

A limitation of the device was found to occur at higher loading frequencies. At the highest loading frequency (40 Hz), there was 5%–8% error (Table II). The loss of accuracy at higher frequencies may have been due to a speed limitation (3000 Hz) of the loading machine software to create an accurate dynamic loading waveform. Also, 40 Hz is approaching the cutoff frequency of the active filters (50 Hz) and some attenuation is possible. However, it may be possible that a PID control system may be needed at these higher frequencies in order to reduce effects of momentum of the thrust rod.

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APPENDIX: BONDING THE STRAIN GAUGE TO RODENT LIMBS

Due to the small size and curved shape of rodent limbs, attachment of a single small gauge is difficult. Following the procedure of others, a Vishay EA-06-015DJ-120 strain gauge is trimmed to an appropriate size,^{5,6,16–18} as shown in Fig. 8. A strain gauge can be trimmed outside the solder pad bonding area (which the manufacturer indicates by a series of dashes and triangles), while the inside is generally kept intact to keep the gauge from falling apart. Yet, with the mouse bones, although not ideal as the gauge can fall apart, it is typically necessary to remove a little of the bonding area to further reduce the size. In the case of the Vishay EA-06-015DJ-120, the size can be reduced from 18 mm² to ~ 1.5 mm².

Bonding the strain gauge to bone requires a sequence of cleaning and preparation. First, the area of bone the gauge will be bonded with is cleaned with ethanol. The back of the strain gauge is coated with activator (Vishay 200 Catalyst-C). A drop of cyanoacrylate (Vishay M-Bond 200) is put on the bone before the bottom of strain gauge is depressed onto and allowing it to cure for 20 min.^{16,18} It is important to add flux

(Vishay M-Flux AR) to all joints (solder pad and wire) to be soldered to remove containments and promote adhesion. The wires should be of small gauge (28–32) and of short length (25 mm) to so that little stress from its weight is added to the pads, which are now more prone to coming unbounded with the backing. Once the solder connections are complete, the wire is protected from stress with the addition of acrylic coating (Vishay Gagekote).

- ¹R. M. Aspden, T. Larsson, R. Svensson, and D. Heinegard, *J. Biomed. Eng.* **13**, 521 (1991).
- ²T. A. Sikoryn, R. M. Aspden, and D. W. Hukins, *J. Biomed. Eng.* **10**, 357 (1988).
- ³M. M. Saunders and H. J. Donahue, *Med. Eng. Phys.* **26**, 595 (2004).
- ⁴J. A. O'Connor, L. E. Lanyon, and H. MacFie, *J. Biomech.* **15**, 767 (1982).
- ⁵A. G. Robling and C. H. Turner, *Bone* **31**, 562 (2002).
- ⁶J. C. Fritton, E. R. Myers, T. M. Wright, and M. C. van der Meulen, *Bone* **36**, 1030 (2005).
- ⁷T. S. Gross, S. Srinivasan, C. C. Liu, T. L. Clemens, and S. D. Bain, *J. Bone Miner. Res.* **17**, 493 (2002).

- ⁸D. J. Webster, P. L. Morley, G. H. van Lenthe, and R. Muller, *Comput. Methods Biomech. Biomed. Eng.* **11**, 435 (2008).
- ⁹S. M. Tanaka, I. M. Alam, and C. H. Turner, *FASEB J.* **17**, 313 (2003).
- ¹⁰P. Zhang, Q. Sun, C. H. Turner, and H. Yokota, *J. Bone Miner. Res.* **22**, 1979 (2007).
- ¹¹A. B. Castillo, I. Alam, S. M. Tanaka, J. Levenda, J. Li, S. J. Warden, and C. H. Turner, *Bone* **39**, 1087 (2006).
- ¹²C. H. Turner, M. P. Akhter, D. M. Raab, D. B. Kimmel, and R. R. Recker, *Bone* **12**, 73 (1991).
- ¹³M. P. Akhter, D. M. Cullen, E. A. Pedersen, D. B. Kimmel, and R. R. Recker, *Calcif. Tissue Int.* **63**, 442 (1998).
- ¹⁴M. R. Forwood, M. B. Bennett, A. R. Blowers, and R. L. Nadorfi, *Bone* **23**, 307 (1998).
- ¹⁵M. D. Roberts, *J. Biomech.* **42**, 2431 (2009).
- ¹⁶R. L. De Souza, M. Matsuura, F. Eckstein, S. C. Rawlinson, L. E. Lanyon, and A. A. Pitsillides, *Bone* **37**, 810 (2005).
- ¹⁷S. J. Warden and C. H. Turner, *Bone* **34**, 261 (2004).
- ¹⁸M. Su, H. Jiang, P. Zhang, Y. Liu, E. Wang, A. Hsu, and H. Yokota, *Ann. Biomed. Eng.* **34**, 1600 (2006).
- ¹⁹N. Ohashi, A. G. Robling, D. B. Burr, and C. H. Turner, *J. Bone Miner. Res.* **17**, 284 (2002).