Time-dependent behaviour of demineralised trabecular bone – experimental investigation and development of a constitutive model

Time-dependent behaviour of demineralised trabecular bone – experimental investigation and development of a constitutive model

Shuqiao Xie[1], Robert J. Wallace[2], Pankaj Pankaj[1*]

[1]School of Engineering, Institute for Bioengineering, The University of Edinburgh, Alrick Building, The King's Buildings, Edinburgh EH9 3BF, UK

[2]Department of Orthopaedics, The University of Edinburgh, Chancellor's Building, Edinburgh EH16 4SB, UK

*** Correspondence:**
Professor Pankaj Pankaj
pankaj@ed.ac.uk

Abstract

Trabecular bone is a cellular composite material comprising primarily of mineral and organic phases and its mechanical response to loads is time-dependent. The contribution of the organic phase to the time-dependent behaviour of bone is not yet understood. We investigated the time-dependent response of demineralised trabecular bone through tensile multiple-load-creep-unload-recovery experiments. We found that demineralised trabecular bone's time-dependent response is nonlinearly related to the applied stress levels - it stiffens with increased stress levels. Our results also indicated that the time-dependent behaviour is associated with the original bone volume ratio (BV/TV). Irrecoverable strain exists, even at the low strain levels, but are not associated with BV/TV. Furthermore, we found that the nonlinear viscoelastic model can accurately predict the time-dependent behaviour of the trabecular bone's organic phase, which can be incorporated together with the properties of mineral to generate a composite model of bone. This study will help to provide a better understanding of this natural composite material.

Keywords: Bone volume ratio, recoverable and irrecoverable strain, nonlinear viscoelasticity, collagen, creep compliance.

1 Introduction

Bone has been known to be a composite material which comprises of a mineral phase (mainly carbonated hydroxyapatite), organic phase (mostly type I collagen) and water assembled into a complex, hierarchical structure (Currey 1964). Currey (1969) also described the time-independent mechanical properties of bone (e.g. elastic modulus) as a function of its ash content and showed that it increases with increasing mineralisation. However, since bone is not an isotropic material its mineral density is not the only determinant of its elastic modulus. This was confirmed by Bonfield and Li (1967) who reported a variation in Young's modulus with orientation. Later, a two-level hierarchical fibre-reinforced composite model of bone was developed by Katz (1980) to quantify the effect of orientation. Literature shows that the subject of developing the time-independent elastic properties of bone as a function of its constituents has been a subject of research for the past five decades.

The organic phase of bone can be isolated through a demineralisation process, which generally comprises of submerging bone samples in a chemical solution, e.g. ethylenediaminetetraacetic acid (EDTA) or hydrochloric acid (HCl). One of the earliest studies on the mechanical properties of demineralised bone was by Burstein et al. (1975), who examined the tensile mechanical behaviour of progressively demineralised cortical bone obtained from bovine tibia by using HCl solution of varying concentration (0.005-0.5N); the authors showed that the ultimate stress, yield stress and Young's modulus decreased progressively with increasing HCl solution concentration. Other studies on demineralised bone examined its elastic behaviour (Bowman et al. 1996; Catanese et al. 1999; Chen and McKittrick 2011; Novitskaya et al. 2011) and its cyclic behaviour (Novitskaya et al. 2013). A number of studies have shown a nonlinear load-deformation response from monotonic loading experiments (Bowman et al. 1996; Catanese et al. 1999; Novitskaya et al. 2011) and cyclic loading tests (Novitskaya et al. 2013; Xie et al. 2018b). However, studies that have considered time-dependent behaviour of demineralised trabecular bone have been limited (Bowman et al. 1994).

It has been widely recognised that the mechanical response of bone, when subjected to loads, is not instantaneous, but is time-dependent (Bowman et al. 1994; Manda et al. 2016, 2017; Xie et al. 2017). However, the contribution of the constituent components of bone to its time-dependent behaviour has received little attention. Bowman et al. (1999) investigated the creep behaviour of fully demineralised cortical bone at varying normalised stresses and reported that the samples possessed three classical regimes of creep – primary, secondary and tertiary. Although this study considered varying load

levels, it achieved this by applying different load levels to different samples, i.e. each sample was subjected to a single load level only. By conducting fully reversed cyclic tension-compression loads with varying load on demineralised trabecular bone samples, asymmetric behaviour – stiffening in tension and softening in compression – was observed (Xie et al. 2018b). However, this cyclic experimental protocol could not be used readily for the development of time-dependent constitutive models.

In summary, while there have been a number of studies to examine the time-independent behaviour of demineralised bone (Bowman et al. 1999; Catanese et al. 1999; Chen and McKittrick 2011; Novitskaya et al. 2011), studies examining its time-dependent response are lacking (Bowman et al. 1996; Xie et al. 2018b). Therefore, the primary objectives of this study were as follows. Firstly, to experimentally evaluate the time-dependent behaviour of demineralised trabecular bone by undertaking creep and recovery experiments at multiple load levels. Secondly, to describe the experimental results using time-dependent constitutive models. Lastly, by performing micro-computed tomography (μCT) on the samples prior to demineralisation, evaluate how the response is influenced by the original bone volume ratio (BV/TV).

2 Materials and methods

2.1 Sample preparation

Four fresh bovine proximal tibias under 30 months old when slaughtered were obtained from a local abattoir and stored at -20°C until utilised. The bones were allowed to thaw at room temperature before bone cores were extracted along its principal axis, using diamond coring tools with an inner diameter of 10.7 mm (Starlite, Rosement, IL, USA). A low speed rotating saw (Buehler, Germany) was used to create parallel sections and to trim growth plates if they were present. All coring and cutting was conducted in a water bath to reduce heat generation. The cylindrical bone samples (n=7) had a mean height of 20.4 ± 0.7 mm.

Bone marrow was removed from each sample using a dental water jet (Interplak, Conair) with tap water at room temperature (Lievers et al. 2007). All the samples were then centrifuged at 2000 r.p.m for 2 hours to remove any residual marrow (Sharp et al. 1990). All the samples were scanned using μCT scanner (Skyscan 1172, Bruker, Kontich, Belgium) at a resolution of 17.22 μm and the system's software (CTan, v1.13.5.1) was used to evaluate the bone volume to total volume ratio (BV/TV) of the bone, which was found to be in the range 15.5 - 37.6 %. Scanning parameters used were: source

voltage 54 kV, current 185 μA, exposure 885 ms with a 0.5 mm aluminium filter between the X-ray source and the sample. The image quality was improved by using 2 frames averaging. It should be noted that the BV/TV mentioned in this study relates to the bone volume ratio of the sample prior to demineralisation.

2.2 Demineralisation

After scanning, demineralisation was conducted by submerging samples in 20 ml 0.6N hydrochloric acid (HCl) at room temperature. The tubes were loaded into a wire rack to secure and vertically orientate them, before being placed in an ultrasonic tank. Ultrasonic agitation was applied at a frequency of 20kHz in order to increase the rate of demineralisation (Wallace et al. 2013). Hydrochloric acid has been successfully used for demineralising bone in previous studies (Burstein et al. 1975; Chen et al. 2011; Chen and McKittrick 2011; Castro-Ceseña et al. 2013; Xie et al. 2018b). The solution was changed daily (Chen and McKittrick 2011) for two weeks after which the completeness of demineralisation was verified using μCT scanning. All samples in this study were found to be fully demineralised in 2 weeks.

2.3 Mechanical testing

Samples were fixed into end-caps using bone cement (Simplex, Stryker, UK) with the assistance of a custom made alignment tool in order to minimise the end-artefacts during testing (Keaveny et al. 1997). Bone cement had a Young's modulus above 1 GPa which is much higher than the modulus of demineralised bone sample. Therefore, the strain response of bone cement layer can be assumed to be negligible in comparison to that of the sample. The effective length (17.4 ± 0.7 mm) of each sample was calculated as the exposed length of the sample between the end-caps plus half the length of the sample embedded within the end-caps (Keaveny et al. 1997). Each sample was placed in an epoxy tube filled with phosphate-buffered saline (PBS) solution to ensure that they remained hydrated during all stages of mechanical testing (Xie et al. 2018b) as shown in Fig. 1.

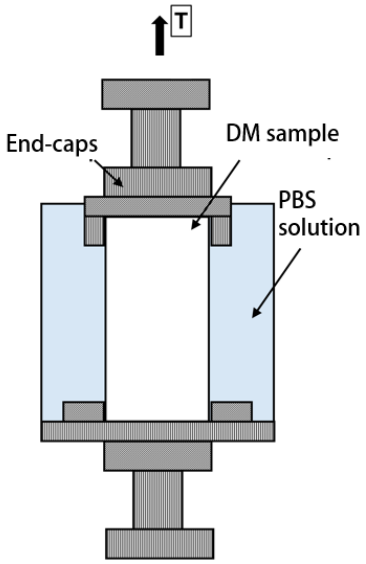
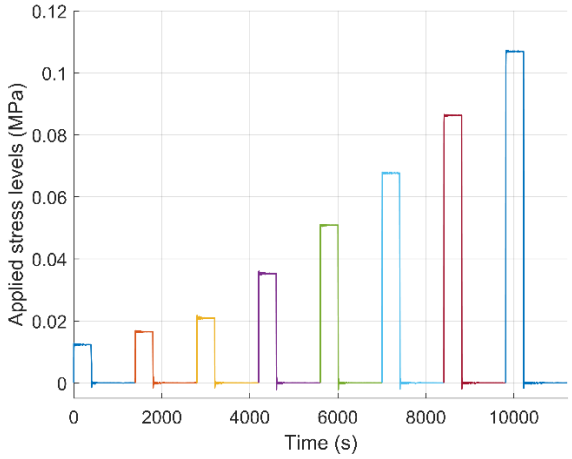


Figure 1: Schematic representation of loading set up. Samples are kept hydrated in a PBS solution throughout mechanical testing

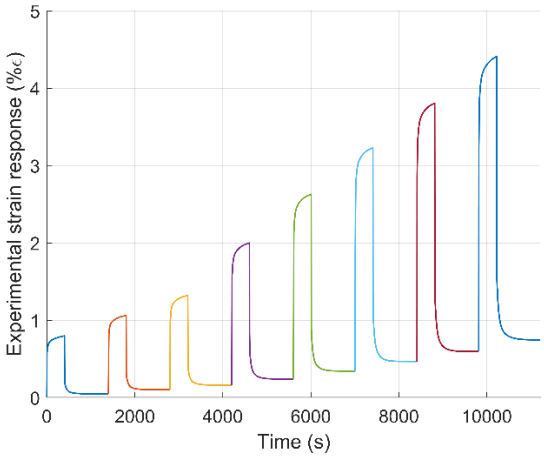
Each sample was first preconditioned by subjecting it to 10 cycles of tensile loading with an amplitude of 0.1 % apparent strain (Xie et al. 2017). The tensile multiple-load-creep-unload-recovery (MLCUR) experiment was conducted on 7 fully demineralised trabecular bone samples using Instron material testing machine (50N load cell, Model 3367) at room temperature. These tests comprised of cyclic loading in which samples were loaded, permitted to undergo creep, unloaded and then left to recover.

Bone has been shown to yield at relatively isotropic strains as compared to stresses, and the yield strain is not dependent upon apparent elastic stiffness or density (Vahey et al. 1987; Levrero-Florencio et al. 2016). Consequently strain-based loading cycles were applied. Loading cycles comprised static loading strain of 0.6 %, 0.8 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, 3.0 % and 3.5 % apparent static strains at the rate of $0.01s^{-1}$. Loading and unloading phases were under displacement control. The chosen strain rate has been successfully used previously to characterise the time-dependent behaviour of trabecular bone (Manda et al. 2016, 2017; Xie et al. 2017). When the designated target strain was achieved, the corresponding load was maintained for 400 s, thereby permitting the sample to undergo creep. For a typical sample, (medium porosity, BV/TV = 26.8 %) corresponding load levels are shown in Fig. 2a. Each loading step was followed by an unloading step to a zero force at the same rate used for loading ($0.01\ s^{-1}$) and this zero force was maintained for 1000s before proceeding to the next cycle (Fig. 2a). These durations for creep and recovery were

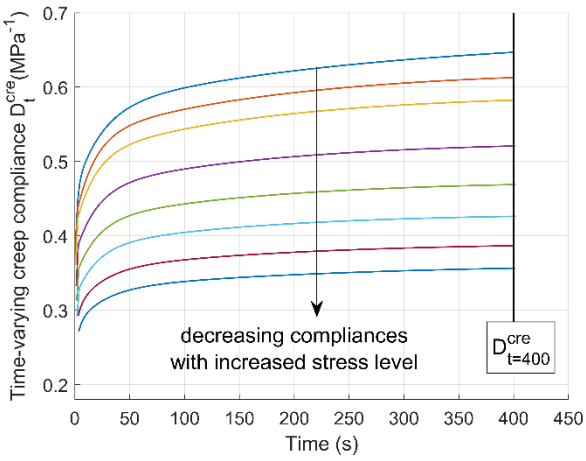
133 determined after initial pilot tests which showed that 400 s and 1000 s were more than sufficient for
134 the samples to achieve a constant creep rate and for recovery curves to reach a plateau, respectively.
135 The dataset for these demineralised trabecular bone samples under tensile multiple-load-creep-unload
136 experimental are available from Edinburgh DataShare (Xie et al. 2018a).



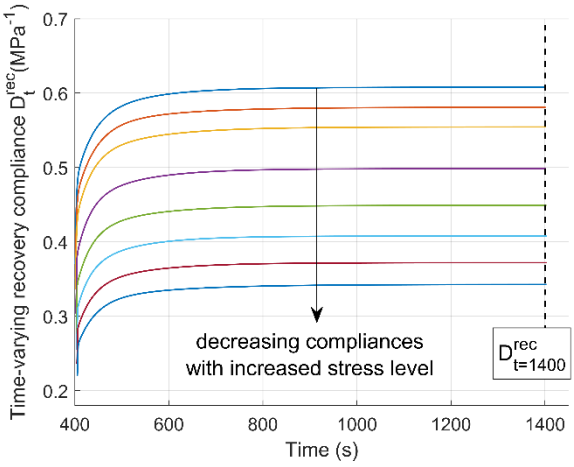
(a)



(b)



(c)



(d)

Figure 2: Response of a typical sample (BV/TV = 26.8%) subjected to tensile multiple-load-creep-unload-recovery cycles. Load application (a); strain response (b); time-varying creep compliance (c) and recovery compliance (d) at varying stress levels. In each cycle, plateau load was held constant for 400 s and strain recovery was measured for another 1000s before next cycle application. The applied load in each cycle corresponds to the static strain of 0.6 %, 0.8 %, 1.0 %, 1.5 %, 2.0 %, 2.5 %, 3.0 % and 3.5 %

2.4 Material model

We used both linear on nonlinear viscoelasticity models to describe the time-dependent behaviour of trabecular bone. The linear Kelvin-Voigt model or Prony series was employed to describe the time-varying compliance, *D(t)*, given by

$$D(t) = D_g + \sum_{i=1}^{n} D_i[1 - \exp(-t/\tau_i)] \quad (1)$$

where D_g is instantaneous compliance that describes the elastic response at time t=0, D_i are transient retardation strengths associated with retardation times (τ_i). The nonlinear viscoelastic model was based on the approach of Park and Schapery (1999) in which the time-varying compliance includes nonlinear stress-dependent parameters, g_0, g_1, g_2 and α_σ , and is given by

$$D(t) = g_o D_g + g_1 g_2 \sum_{i=1}^{n} D_i[1 - \exp(-\frac{t}{\alpha_\sigma}/\tau_i)] \quad (2)$$

Following the approach used for untreated (or non-demineralised) bone (Manda et al. 2017), where the unloading phase from the first cycle was assumed linear viscoelastic, the glassy or instantaneous compliance (D_g), transient retardation strengths (D_i) and retardation times (τ_i) were evaluated by minimising the errors between experimental measurements and Eq. 1. A three-term Prony series (n=3) was chosen which gave a fitting error of less than 0.3%. The parameter g_0 is a nonlinear instantaneous compliance parameter, the transient nonlinear parameter g_1 measures the effect of nonlinearity in the transient compliance, g_2 describes the effect of loading rate on transient creep response, and α_σ is a time-shift factor. Thereafter, the stress dependent nonlinear parameters g_2 and α_σ in Eq. 2 were evaluated first by using the recovery strain response from cycles 2 to 8, followed by evaluation of parameters of g_0 and g_1 using the entire unloading phase from each cycle (Manda et al. 2017). The nonlinear parameters obtained from each stress level were then expressed as smooth second-order polynomial functions of stresses.

3 Results

Without exception, each sample exhibited classical rapid primary and slow secondary regimes of creep behaviour across all stress levels. All 7 samples could be subjected to stress levels corresponding to the highest designated strain level (3.5 %) without tertiary creep or failure.

3.1 Experimental observations

Figure 2b shows a strain response obtained from our tensile multiple-load-creep-unload-recovery (MLCUR) experiment for one typical sample (medium porosity, BV/TV = 26.8 %). For this sample the stress levels varied between 12.3 *kPa* and 106.9 *kPa* for the minimum and maximum applied strain levels of 0.6 *%* and 3.5 *%*, respectively. The time-varying compliance, defined as the ratio between time-varying strain and its corresponding stress level, was evaluated for both creep and recovery phases, denoted as D_t^{cre} and D_t^{rec} respectively. Both time-varying compliances were found to increase with time for all stress levels, as would be expected for a viscoelastic material (Fig. 2c and 2d). For linear viscoelastic materials, the time-varying compliance curves would be identical for all stress levels. However, the time-varying compliances, D_t^{cre} and D_t^{rec}, derived for demineralised trabecular bone were found to vary with applied stress levels, indicating a nonlinear response.

It was found that the time-varying compliance decreases with increasing stress levels (i.e. the curves at lower stress levels are above those at the higher stress levels), and this is true for both creep and recovery compliances (Fig 2c and 2d, respectively for the typical sample considered). This decreasing trend was followed by all the samples tested, which demonstrates elastic stiffening with increasing stress levels. It is also observed that the time-varying recovery compliance (D_t^{rec}) was somewhat smaller than creep compliance (D_t^{cre}) in each corresponding cycle. It was apparent that the creep compliance (D_t^{cre}) showed an increasing trend with time for all loading cycles, whereas the recovery compliance (D_t^{rec}) reached a plateau after around 400 s of recovery. This indicates that while irrecoverable strain develops in the loading and load holding phases, only viscoelastic strain is recovered during unloading and recovery phases.

Compliance at the end of creep (t = 400 s) and recovery (t = 1400 s) from every loading cycle was obtained (for example see Fig. 2c and 2d for the sample with BV/TV = 26.8%) and denoted as $D_{t=400}^{cre}$ and $D_{t=1400}^{rec}$, respectively. This provided eight values of $D_{t=400}^{cre}$ and eight values $D_{t=1400}^{rec}$ for every sample. Figure 3 shows both compliances plotted against normalised stress (σ/σ_o) defined as the stress applied in each cycle divided by the stress applied in the first cycle (Manda et al. 2017) for all the samples tested.

The creep compliance at t = 400 s ($D_{t=400}^{cre}$) was in the range of 0.24 to 1.40 MPa^{-1}. Recovery compliance at t = 1400 ($D_{t=1400}^{rec}$) was in the range of 0.22 to 1.35 MPa^{-1} as shown in Fig. 3. The decreasing trend in compliances with increased normalised stress level was observed for all tested

samples. These results also showed that the samples with higher BV/TV have, in general, lower compliance in comparison to samples with lower BV/TV. However, samples with BV/TV = 33.8 % and 23.8 % were found to be exceptions to this trend which cannot be readily explained. We believe that the micro-architectural orientation may have played a much stronger role for these two demineralised samples. For untreated (non-demineralised) bone it has been shown that bone mineral density (or indirectly BV/TV) which is the primary method to identify bone quality, does not often correlate with mechanical behaviour well (Schuit et al. 2004) and the porous microarchitecture of bone is an important determinant of its mechanical behaviour (Homminga et al. 2002). For the sample with BV/TV=23.8%, which has an extremely low compliance, it is also possible that during the experiment excessive cement was used which permeated through the sample. Therefore, we have excluded this sample from subsequent analysis.

Decreasing compliance with increased stress level indicates that the demineralised trabecular bone behaves in a nonlinear manner and it stiffens with increasing stress levels. Therefore, the use of a nonlinear constitutive model is required to describe the time-dependent behaviour of demineralised trabecular bone.

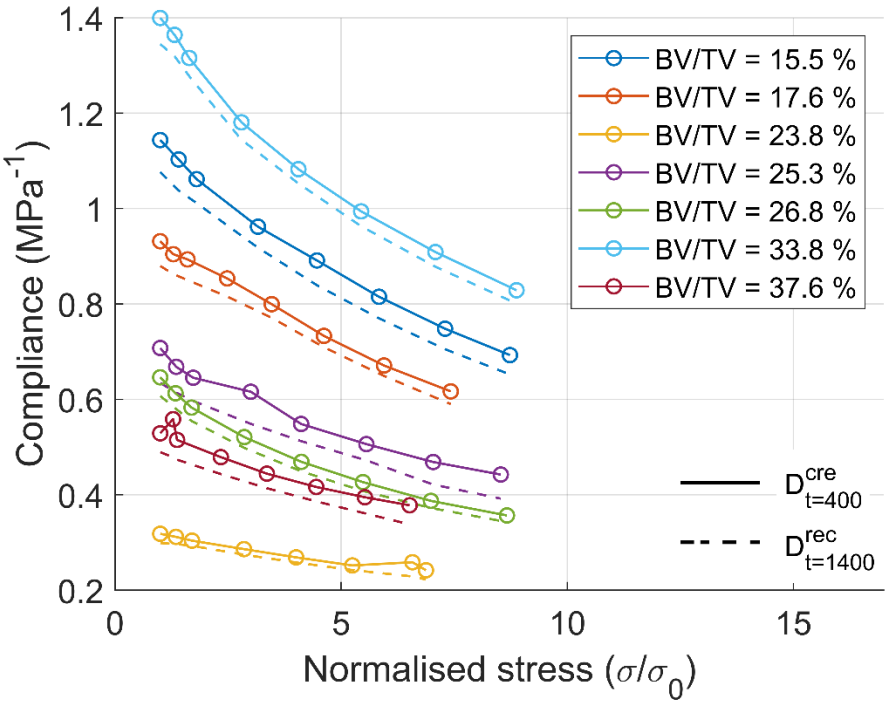


Figure 3: Time-varying creep compliance at 400 s ($D^{cre}_{t=400}$) and recovery compliance at 1400 s ($D^{rec}_{t=1400}$) plotted against normalised stress for all 7 samples

The compliances from the recovery regimes become constant (recovery curve reaches a plateau) in a short time, which indicates that the viscoelastic strain is perhaps completely recovered in the 1000 s recovery time provided in all cycles. The strains present at the end of each cycle were therefore deemed irrecoverable. This irrecoverable strain was found to exist even at the end of the first loading cycle corresponding to the smallest load level. Figure 4 shows the irrecoverable strain along with its applied static strain for all the cycles and all the samples. A power-law relationship ($r^2 = 0.75$, $p < 0.0001$) was found between irrecoverable strain and applied static strain. However, no significant correlation was found between the irrecoverable strain and the original BV/TV (Fig. 4). It is clear that this irrecoverable strain increases with increasing applied load level for all demineralised trabecular bone samples.

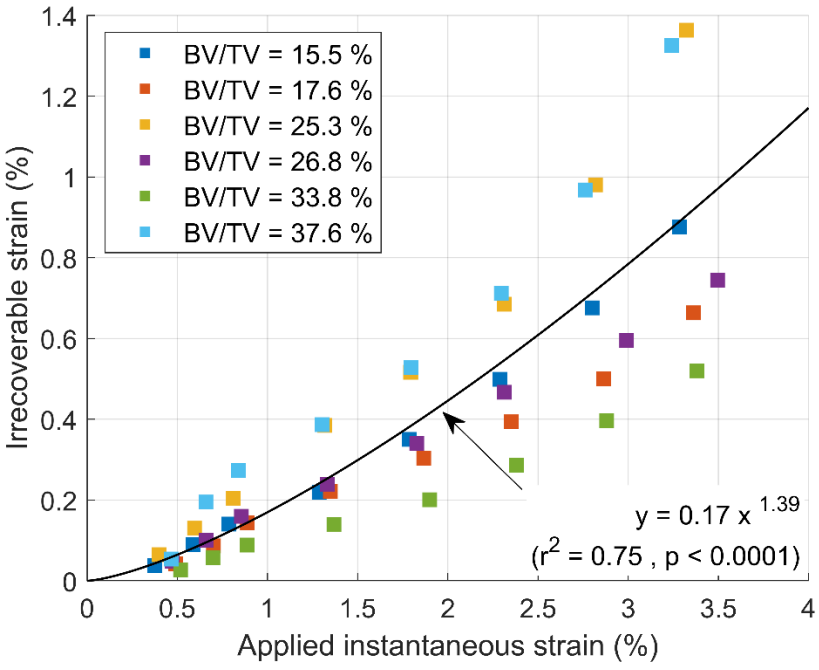


Figure 4: Irrecoverable strain at the end of each loading cycle for all the samples plotted against the applied instantaneous strain (where plateau force was held constant during the test) with a power-law relationship

3.2 Constitutive model

The instantaneous compliance (D_g) was found to be in the range of 0.33 to 0.66 MPa^{-1} (Fig. 5). It was also found that D_g decreases with increasing BV/TV with a power-law relationship (Fig. 5)

$$D_g = 7.4 \text{ x } (BV/TV)^{-0.91} \ (r^2 = 0.90) \quad (3)$$

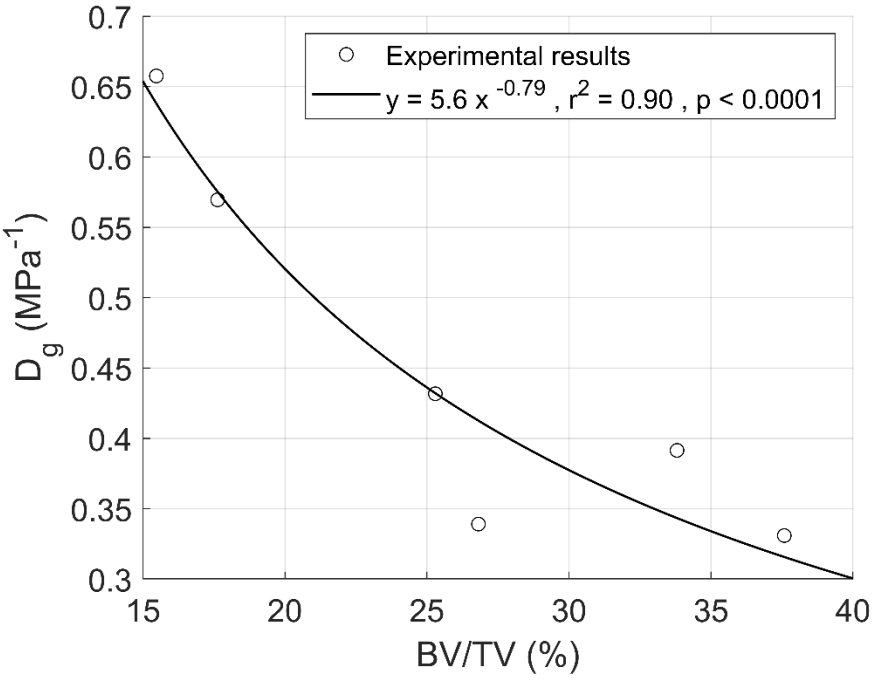
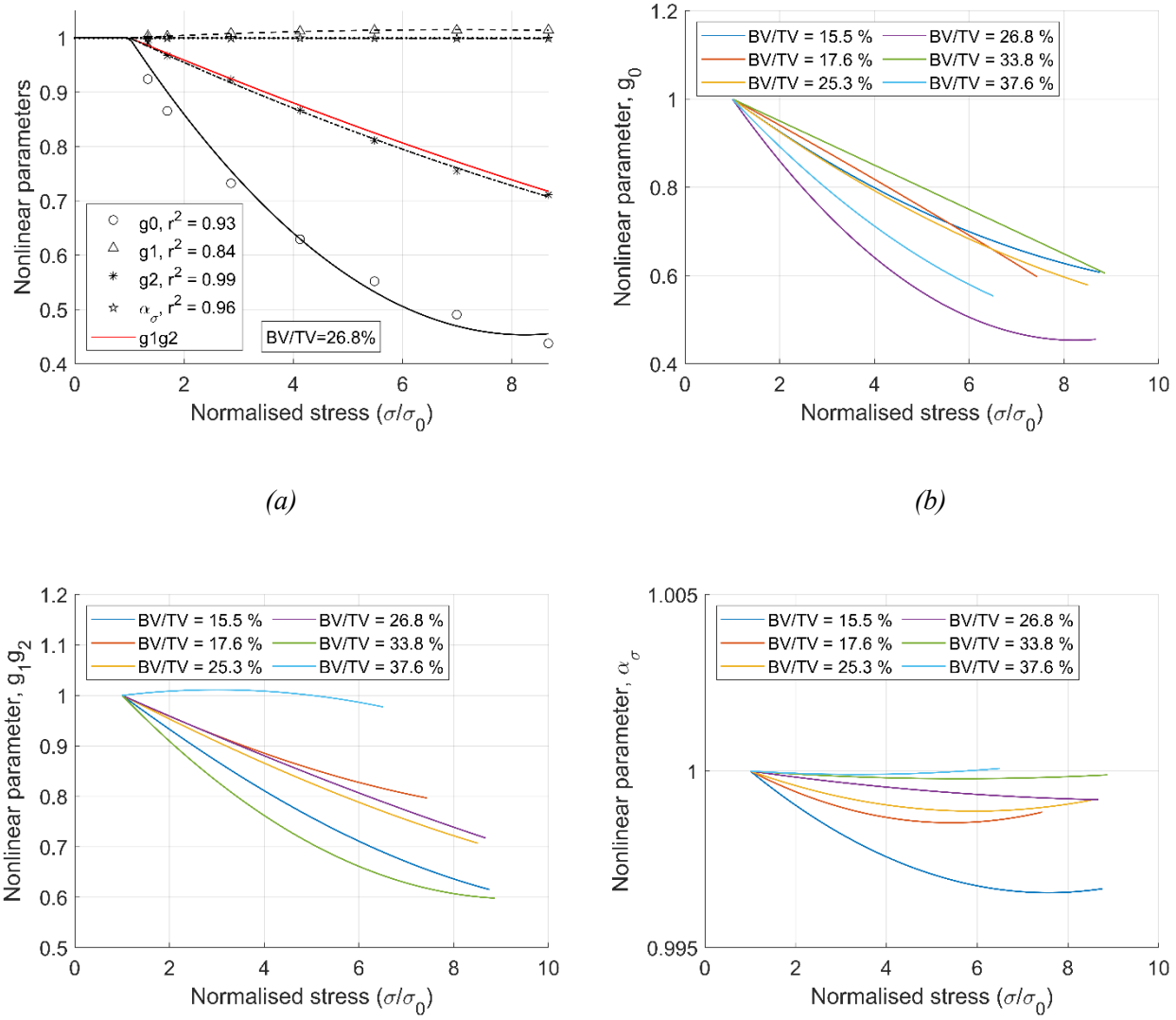


Figure 5: Instantaneous compliance (D_g) plotted against BV/TV with a power law relationship.

Figure 6a shows the variation for the typical sample considered (BV/TV = 26.8%), the values of g_0 and g_2 decreased with increasing normalised stress (σ/σ_o), whereas the values of g_1 and α_σ were almost constant (Fig. 6a). The product of $g_1 g_2$, which affects the transient response was also found to decrease with increasing normalised stress. These observations led to the choice of a second-order polynomial function to represent the nonlinear viscoelastic parameters as functions of normalised stress, which produced coefficients of determination of r^2 = 0.93, 0.84, 0.99 and 0.96 for parameters g_0, g_1, g_2 and α_σ respectively (Fig. 6a). The decreasing trend of curves indicates that the demineralised trabecular bone stiffens with increased stress levels, and this elastic stiffening phenomenon was observed for all the samples tested. The nonlinear parameters for the 6 samples analysed are shown in Fig. 6b-6d and expressed as a second-order polynomial function with normalised stress. It can be seen that the variation described for the typical sample is largely followed by all. The decreasing trend of g_0 and $g_1 g_2$ demonstrates that the demineralised trabecular bone sample experiences elastic stiffening in tension with increasing stress levels. The time-shift factor, α_σ, was found to remain almost constant for all samples.



(a) *(b)*

(c) *(d)*

Figure 6: Nonlinear viscoelastic parameters for the typical sample (a) and parameters for all 6 samples are expressed as second order polynomial functions of normalised stress: g_0 (b), g_1g_2 (c) and α_σ (d).

The accuracy of linear viscoelastic (LVE) and nonlinear viscoelastic (NVE) constitutive models was compared with the experimental data. Figures 7a and b compare the experimentally obtained strain response to that obtained using LVE and NVE predictions for two representative samples with BV/TV = 15.5% and 37.6%. These samples had the lowest and highest BV/TV. For clarity, only the first 5 loading cycles are shown in Fig. 7. It can be seen that both models provide good predictions for the first loading cycle, however, at higher stress levels the NVE and LVE responses deviate from

271 the experimental curves. This is not unexpected as both LVE and NVE models are elastic models,
272 with NVE taking stress dependent nonlinearity into account. We have shown that the samples have
273 irrecoverable strains which elasticity models (linear or nonlinear) cannot incorporate. Figures 7a and
274 b also show irrecoverable strains (denoted as VP or viscoplastic). The viscoplastic (VP) strain was
275 evaluated by taking the difference between creep (which includes both viscoelastic and viscoplastic
276 strains) and recovery curves (viscoelastic strains only). In Fig. 7c and d, the irrecoverable portion of
277 the strain was removed from the experimental curve and it was then plotted against predictions of the
278 LVE and NVE models. It is clear that the NVE constitutive model provides a more accurate
279 prediction of the viscoelastic behaviour of demineralised bone, whereas the LVE constitutive model
280 only gives a reasonable prediction at low loading cycles. The LVE model over-predicts the strain
281 response at the higher stress levels, which also indicates that the demineralised trabecular bone
282 undergoes elastic stiffening at higher stress levels.

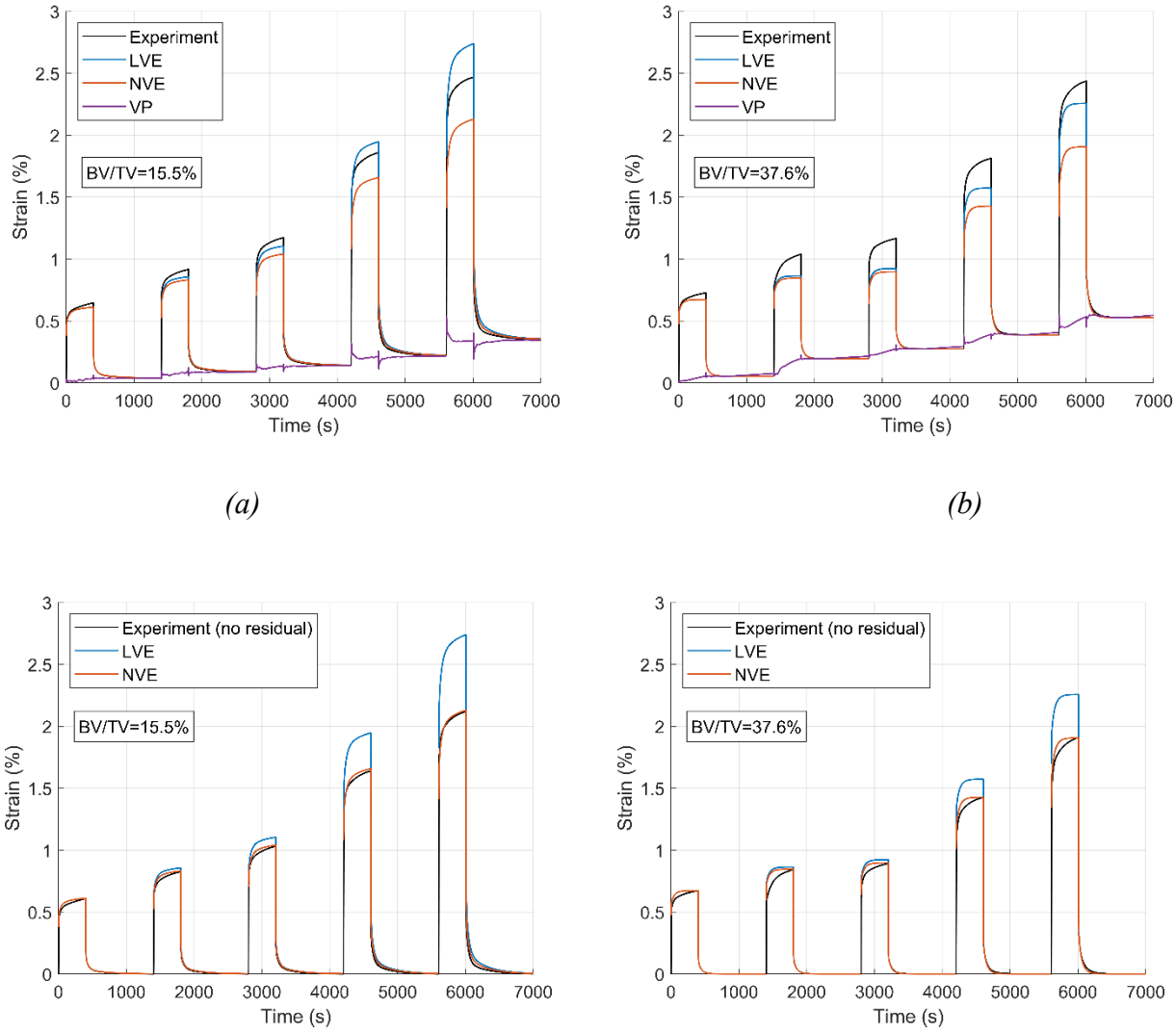


Figure 7: The comparison of experimental strain response and constitutive model predictions. Experimental response and the predicted LVE, NVE strain response and viscoplastic strain (a & b), experimental strain response (with residual strain excluded) compared with predicted linear and nonlinear viscoelastic strain response (c & d), for two samples with lowest and highest BV/TV considered.

4 Discussion

As stated in the introduction, untreated (non-demineralised) trabecular bone has been studied extensively for its time-independent properties and there have also been a number of studies to investigate its time-dependent behaviour through creep, relaxation and dynamic loading.

296 Demineralised bone has been primarily examined for its time-independent properties and studies on
297 its time-dependent behaviour have been extremely limited.

298 Tensile multiple-load-creep-unload-recovery (MLCUR) experiments on demineralised trabecular
299 bone undertaken in this study show that its response to mechanical forces is time-dependent and the
300 strain includes recoverable and irrecoverable components, even at low-stress levels. The results also
301 show that this time-dependent behaviour of demineralised trabecular bone varies nonlinearly with the
302 applied stress. The nonlinear viscoelastic model can predict demineralised trabecular bone's time-
303 dependent behaviour well if the irrecoverable strain is excluded. As found in previous time-
304 independent monotonic loading studies our results also show that demineralised trabecular bone
305 stiffens at increased stress levels.

306 The MLCUR experiments have been previously used to investigate untreated trabecular bone's time-
307 dependent behaviour in compression (Manda et al. 2016, 2017; Xie et al. 2017). This study employed
308 a similar approach to evaluate the time-dependent behaviour of demineralised trabecular bone in
309 tension. We assumed that the first strain level of 0.6% to be in the linear elastic range. Bowman et al
310 (1996) showed that demineralised bone does not yield up to an apparent strain of 10% in tension
311 (Bowman et al. 1996) and that creep failure strains are in the range 8.8-21.1% (Bowman et al. 1999).
312 In fact, Bowman et al. (1999) applied preconditioning cycles between 0-5% strain before applying a
313 loading ramp to obtain linear region of the stress-strain curve. Predictions made using the LVE model
314 indicate that this assumption is accurate for slightly higher strains as well (e.g. see second load cycle
315 in Fig. 7).

316 The observed creep compliances for demineralised trabecular bone in tension were in the range 0.22
317 to 1.40 MPa^{-1} while the creep compliance in compression for untreated bone have been found to be in
318 the range 1.08 $\times 10^{-3}$ to 4.17×10^{-3} MPa^{-1} (Manda et al. 2016). As expected, this indicates that the
319 demineralised trabecular bone is much more flexible than untreated bone. From our experiments the
320 long-term modulus of demineralised bone was calculated by taking reciprocal of the instantaneous
321 compliance (D_g). The evaluated modulus was found to be of similar order as in previous studies for
322 demineralised cortical bone (Burstein et al. 1975; Bowman et al. 1996; Catanese et al. 1999;
323 Novitskaya et al. 2011) and demineralised trabecular bone (Chen et al. 2011; Xie et al. 2018b).
324 However, above-cited studies were all limited to monotonic or cyclic loading.

Bone is recognised as time-dependent material in which the strain (or stress) response to a force (or displacement) is not instantaneous. Similar to untreated trabecular bone (Manda et al. 2017; Xie et al. 2017), demineralised trabecular bone's time-dependent behaviour is nonlinearly related to the applied stress level. Our MLCUR experiments show that at higher stress levels the strain response is smaller than what would be expected if a linear viscoelastic model were used. This indicates that demineralised bone stiffens at higher stress levels. This result is in agreement with previous monotonic loading studies in which the well-known J-shaped stress-strain curve has been observed, not only for demineralised bone (Bowman et al. 1996; Xie et al. 2018b) and actin networks (Schmoller et al. 2010) but also for untreated bone (Kim et al. 2011; Xie et al. 2017). The explanation for the J curve is that initially the kinks in the collagen are straightened out then the collagen fibres start carrying the loads.

Bone volume ratio (BV/TV) or porosity of the bone has been used extensively and successfully to describe bone's time-independent properties (Carter and Hayes 1977; Currey 1988; Gibson and Ashby 1999; Gibson 2005). Some previous study have also used BV/TV in the study of bone's time-dependent behaviour (Manda et al. 2016; Xie et al. 2017). This study shows that time-dependent response of demineralised bone is also strongly associated with BV/TV of the samples prior to demineralisation. Both creep and recovery compliance values largely follow the BV/TV trends (see Fig. 3). As would be expected, samples with higher BV/TV generally have lower compliance.

Our experiments show that unloading results in some irreversible strains at the end of each loading cycle, which increases with increasing stress levels. However, we did not find an apparent relationship between irrecoverable strain and BV/TV. The post-elastic mechanical behaviour is likely to be much more strongly linked to micro-architecture than simply BV/TV of the samples. Reasons for apparent plastic behaviour of bone and demineralised bone have been discussed in previous studies. By conducting uniaxial nano-mechanical compression on cylindrical samples Tertuliano and Greer (2016) proposed that inter-fibrillar sliding through shear of extra-fibrillar matrix was the mechanism of plasticity in bone, which was also suggested by Gupta et al. (2005, 2006). This irrecoverable strain could also be due to inter-fibrillar sliding at nano-scale. This irrecoverable deformation in collagen needs to be emphasised, as it is generally ignored in two-phase composite models of bone (Lubarda et al. 2012).

Composite models of bone can help illustrate the complex interrelationship between bone microstructure and material properties of constituents. In particular, bone is frequently modelled as a

two-phase composite, hydroxyapatite mineral crystals dispersed in an organic matrix. This study shows that the time-dependent behaviour of bone's organic phase can be described using a nonlinear viscoelastic model, which provides a good prediction for recoverable (elastic) strain response at varying load levels. These developed models can be used in conjunction with the mechanical properties of bone's mineral phase for evaluating the influence of the two phases on the time-dependent mechanical response.

This study suffers from a number of limitations. Firstly, all the tests were conducted at room temperature; creep behaviour has been reported to be temperature-dependent for bone (Bonfield and Li 1968; Bowman et al. 1998), so it is likely that the demineralised trabecular bone's viscoelastic behaviour is also temperature-dependent. Secondly, it is not possible in practice to perform ideal creep-recovery experiments and in the tests conducted, the time interval to loading and unloading is finite (e.g. 1 s to reach 1% strain with our designated strain rate 0.01 s^{-1}). Small viscoelastic deformations are likely to occur during loading and unloading.

In conclusion, this study shows that the response of demineralised trabecular bone samples to mechanical forces is time-dependent and it is nonlinearly related to its applied stress levels – it stiffens with increased stress level. Some irrecoverable strain exists even at load cycles corresponding to small strains. Irrecoverable strain, however, is not related to a sample's pre-demineralised BV/TV. The developed nonlinear time-dependent constitutive model can be incorporated together with properties of the mineral phase to generate a composite model of bone.

5 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.

6 Author Contributions

S. Xie: Designed study, conducted experiment, analysed data and drafted manuscript

R. Wallace: Designed study, conducted experiment and revised manuscript critically

P. Pankaj: Designed study, analysed data and revised manuscript critically

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Supplementary information

Constitutive model:

The nonlinear viscoelastic model was based on Manda et al. (2017) which used the Schapery's nonlinear viscoelastic constitutive law (Shapery 1969). In this the strain response to a suddenly applied stress is given by

$$\varepsilon(t) = g_0 D_g \sigma + \mathrm{g}_1 \int_0^t \Delta D(\psi^t - \psi^\tau) \frac{d(g_2\sigma)}{d\tau} d\tau \quad (1)$$

$$\psi^t = \int_o^t \frac{d\tau'}{\alpha_{\sigma(\tau')}\alpha_{T(\tau')}\alpha_{e(\tau')}} \quad (2)$$

where D_g is instantaneous compliance, g_0, g_1, g_2 and α_σ are stress-dependent nonlinear viscoelastic parameters, σ is applied stress and ψ^t is reduced time. Parameters α_σ, α_T and α_e are stress, temperature and other environment time-shift factors, respectively. The effects of temperature (α_T) and other environment variables (α_e) are not considered; consequently, these two parameters are unity. The transient compliance, ΔD, in equation (1) is represented by Prony series as

$$\Delta D(\psi^t) = \sum_1^n D_n[1 - \exp(-\psi^t/\tau_\mathrm{n})] \quad (3)$$

where D_n is *n*th coefficient of the Prony series associated with *n*th retardation time, τ_n. Therefore, the nonlinear time-dependent compliance can be rewritten as

$$D(t) = g_0 D_g + g_1 g_2 \sum_1^n D_n [1 - \exp(-\frac{t}{\alpha_\sigma \tau_n})] \quad (1)$$

This equation reduces to linear viscoelasticity equation if all the stress-dependent nonlinear parameters are unity.

Parameters fitting:

We assume that the first cycle (lowest strain) is linear viscoelastic. Therefore, D_g, D_n and τ_n were evaluated by minimising the errors between experimental measurement and the linear viscoelastic equation. Three Prony terms (n=3) were chosen after a number of fittings and we found that this gave fitting error of less than 0.3%.

The nonlinear parameters, g_2 and α_σ were first evaluated by using the recovery part of the experimental curve. Then the the nonlinear parameters g_0 and g_1 were evaluated by using the entire unloading phase from each cycle. The evaluated nonlinear parameters obtained from each stress level were then expressed as smooth second-order polynomial functions of stresses.

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