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Research paper

Effect of aging on the transverse toughness of human cortical bone: Evaluation by R-curves

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ABSTRACT

The age-related deterioration in the quality (*e.g.*, strength and fracture resistance) and quantity (*e.g.*, bone-mineral density) of human bone, together with increased life expectancy, is responsible for increasing incidence of bone fracture in the elderly. The present study describes *ex vivo* fracture experiments to quantitatively assess the effect of aging on the fracture toughness properties of human cortical bone specifically in the transverse (breaking) orientation. Because bone exhibits rising crack-growth resistance with crack extension, the aging-related transverse toughness is evaluated in terms of resistance-curve (R-curve) behavior, measured for bone taken from a wide range of age groups (25–74 years). Using this approach, both the *ex vivo* crack-initiation and crack-growth toughness are determined and are found to deteriorate with age; however, the effect is far smaller than that reported for the longitudinal toughness of cortical bone. Whereas the longitudinal crack-growth toughness has been reported to be reduced by almost an order of magnitude for human cortical bone over this age range, the corresponding age-related decrease in transverse toughness is merely ~14%. Similar to that reported for X-ray irradiated bone, with aging cracks in the transverse direction are subjected to an increasing incidence of crack deflection, principally along the cement lines, but the deflections are smaller and result in a generally less tortuous crack path.

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1. Introduction

It is widely appreciated that aging-related changes to the musculoskeletal system can lead to a significantly increased susceptibility of bone fracture (Hui et al., 1988; Jennings and de Boer, 1999). In general terms, this has been traditionally attributed to issues of *bone quantity* where the loss in bone mass or bone-mineral density (BMD) from aging is used as a predictor of fracture risk. However, it is now apparent that BMD alone may not be the primary factor responsible for increased bone fractures in the elderly, and that issues associated with *bone quality* may also be important (Hui et al., 1988; Aspray et al., 1996; Heaney, 2003), a fact that has resulted in a renewed interest in how biological factors such as aging can alter the mechanical properties of bone, particularly the fracture resistance.

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Numerous studies that have looked at age-related issues in the mechanical properties of bone have shown a significant deterioration in the bone toughness with age (Burstein et al., 1976; Currey, 1979; Bonfield et al., 1985; Brown and Norman, 1995; Currey et al., 1996; Vashishth et al., 1997; Wang et al., 1998; Zioupos and Currey, 1998; Zioupos et al., 1999; Yeni and Norman, 2000; Brown et al., 2000; Phelps et al., 2000; Wang et al., 2002; Wu and Vashishth, 2002; Nalla et al., 2004; Akkus et al., 2004; Nalla et al., 2006). Many of these have utilized the fracture toughness, K_c, or the strain-energy release rate, G_c, as a single-parameter approach to characterize the resistance to fracture, on the implied assumption that the toughness represents the stress intensity or energy expended to prevent a crack from causing fracture, *i.e.*, the crack-*initiation* toughness. However, the fracture resistance in many materials, particularly biological materials such as bone, can be attributed to microscopic mechanisms that act to inhibit the *growth* of small cracks, *i.e.*, the crack-growth toughness; accordingly, characterization of the fracture resistance requires the use of the so-called crack resistance or R-curve, which defines how the fracture resistance (*e.g.*, K or G) increases with the stable crack extension of small cracks prior to unstable fracture (Vashishth et al., 1997; Nalla et al., 2003). In cortical bone, such mechanisms, termed extrinsic toughening mechanisms,[2] operate at size-scales typically above a micrometer and principally involve such processes as crack deflection (primarily at "cement lines") and crack bridging (by intact regions left in the wake of the crack tip) (Launey et al., 2010; Nalla et al., 2003; Koester et al., 2008).

Consistent with the well-known increase in bone fracture risk with age, R-curve measurements on human cortical bone tested in the *longitudinal* (proximal–distal) orientation (Fig. 1) have shown a distinct deterioration in the individual contributions to the crack-initiation and growth toughness with aging (Fig. 2) (Nalla et al., 2004). This decay in bone quality with aging can be mechanistically associated with increased collagen cross-linking leading to diminished plasticity from fibrillar sliding (the intrinsic effect) and an increase in the osteonal density leading to a diminished role of crack bridging (the extrinsic effect) (Nalla et al., 2004, 2006). However, corresponding aging-related R-curve toughness measurements for the *transverse* orientation in human cortical bone have not been available. In many respects, this is the more critical orientation as it represents the primary breaking, rather than splitting, direction for fractures with the clinical expectation that the transverse toughness would also decrease with age. Here, unlike the longitudinal orientations where crack bridging provides for toughening (Launey et al., 2010; Nalla et al., 2003; Koester et al., 2008), the primary microscale contribution to the bone toughness

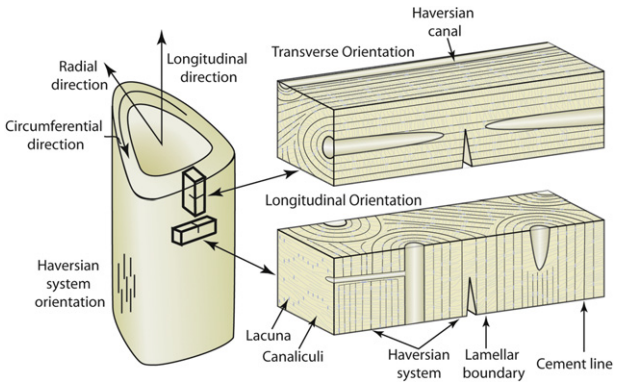

Fig. 1 – Schematic illustrations of the three-point bend samples used for R-curve testing in the transverse and longitudinal (proximal–distal) orientations in relation to the bone-matrix microstructure in human cortical bone.

in the transverse orientation arises from crack deflection and twist, mainly at the cement lines, leading to highly tortuous crack paths and consequently an enhanced fracture resistance (Launey et al., 2010; Koester et al., 2008). With aging, the spacing of these cement lines (associated with an increase in osteonal density) will likely be decreased (Nalla et al., 2006); however, it is uncertain mechanistically whether this will result in a significant change in the incidence of crack deflection, and whether this will significantly affect the crack path and hence the transverse toughness. Accordingly, in this work we specifically examine the measured R-curve fracture toughness and corresponding toughening mechanisms in human cortical bone tested in the transverse orientation as a function of aging to discern whether the transverse toughness is actually affected by age and how mechanistically this could occur.

2. Materials and methods

Test samples from the midsection of frozen cadaveric humeral and tibial cortical bone[3] from six donors were used in this study. The age of these donors, who had no known history of bone-related diseases, were 25, 34, 37, 61, 69, and 74 years old (cause of donor death unrelated to skeletal state); the gender of the donors together with anatomical location are given in Table 1 in the 25–37 years old age group is termed *Young* ($N = 4$), in the 61 and 69 years old age group *Aged* ($N = 5$).[4] In addition, samples were also taken from a male donor 74 years old with diabetes, termed *Aged Diseased* ($N = 3$). Tibiae were used for the 25 and 74 years old donors and humeri were used for the remainder of the donors. All of the donors were male with the exception of the 34 and 69 years old donors.

[2] Fracture can be considered to result from a mutual competition between two classes of mechanisms: intrinsic mechanisms that operate ahead of the crack tip, and affect the material's inherent resistance to fracture and damage, and extrinsic mechanisms that principally operate in the wake of the crack tip, and "shield" the crack from the applied driving force (Ritchie, 1988, 1999; Launey et al., 2010). Whereas intrinsic mechanisms primarily govern the crack-initiation toughness, extrinsic mechanisms, such as crack deflection and crack bridging in bone (25), can only affect the crack-growth toughness.

[3] There can be differences in the cortical structure of humerus and tibial bone in that the latter can show a higher orientation of the collagen fibrils and the mineral platelets that they contain. In the present study, however, this was not reflected in the measured toughnesses in any group.

[4] Results were not obtained from one sample as it was inadvertently damaged prior to testing.

Table 1 – Fracture toughness and R-Curve behavior of human cortical bone with aging (transverse orientation).

Donor information	Sample thickness (mm)	Initiation toughness K_o – MPa$\sqrt{m}$	Growth toughness $dK/d\Delta a$ – MPa$\sqrt{m}/\mu m$	Coeff. of determination (R^2)
Young (25–37 years)				
25MT[a]	2.26	6.30	0.040	0.980
25MT	2.39	4.14	0.042	0.989
34FH7	1.29	5.40	0.028	0.979
37MH7	1.87	6.70	0.033	0.984
Aged (61–69 years)				
61MH	1.42	5.17	0.029	0.960
61MH	1.53	4.48	0.026	0.973
69FH	1.20	6.20	0.036	0.968
69FH	1.37	5.80	0.032	0.976
Diseased Aged (74 years)				
74MT	1.38	4.00	0.028	0.989
74MT	2.04	2.36	0.044	0.994
74MT	1.75	3.24	0.024	0.994

[a]The notation reads as follows: Age (years), Sex (M = Male, F = Female), Extremity (H = humerus, T = Tibiae).

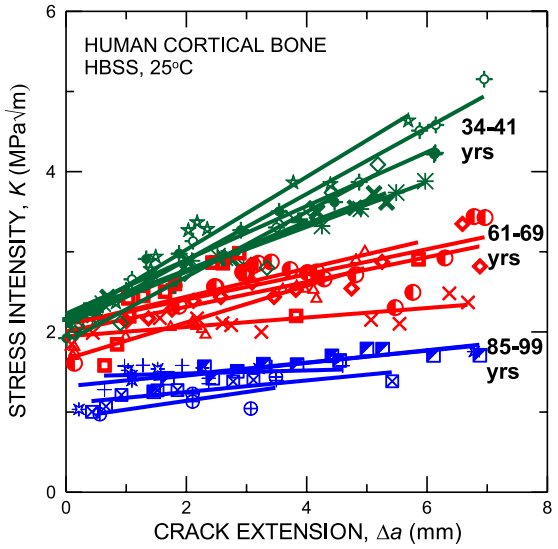

Fig. 2 – Effect of aging on the fracture toughness of human cortical bone in the longitudinal (proximal–distal) orientation, showing $KR(\Delta a)$ resistance curves for stable *ex vivo* (25 °C HBSS) crack extension in human bone in three age groups: *Young* (34–41 years), *Middle-Aged* (61–69 years) and (*Aged*) 85–99 years. Note the factor of two decrease in crack-initiation toughness (defined at $\Delta a \rightarrow 0$) compared to the order of magnitude decrease in crack-growth toughness (slope of the R-curve) with aging. Samples were orientated with the starter notch and the nominal crack-growth direction along the proximal–distal direction of the humerus (in the longitudinal–radial plane), *i.e.*, parallel to the long axis of the osteons and hence, long axis of the humerus.
***Source:* Adapted from Nalla et al. (2004).**

Blocks of bone were obtained by carefully sectioning the medial cortices of the mid-diaphyses of the humeri and tibia using a low speed saw and machined into twelve 1.2–2.4 mm thick,[5] 8 mm long bend samples (width W = 2 mm) for R-curve testing. These samples were then notched to form an initial crack of roughly half the sample width, which was then sharpened with a micro-notching technique by polishing with a razor blade irrigated with 1 μm diamond suspension; resulting root radii were consistently ~10 μm (Ritchie et al., 2008). The location of the samples along the axis of the bone and their corresponding notching was performed so that they were oriented such that the nominal crack-growth direction for subsequent toughness testing was transverse to the long axis of the humerus, *i.e.*, they were in the transverse orientation (Fig. 1). All samples were immersed in ambient Hanks' Balanced Salt Solution (HBSS) for 24 h prior to testing.

Samples for whole bone testing were sectioned from the midshaft of frozen cadaveric femurs using a hacksaw. The donors of the femoral tissue were both male and both of the femurs were from the right leg. The younger donor was 48 years old, 1.9 m tall, and weighed 111 kgf. The older donor was 79 years old, 1.7 m tall, and weighed 68 kgf. The samples were ~150 mm long and 32 mm in diameter, and were notched perpendicular to the long axis of the bone using a hacksaw to cut through the cortical wall; the notch was then sharpened using the same razor-notching technique described above. The whole bone femur samples were immersed in ambient HBSS for 24 h before being tested in HBSS at ~25 °C in three-point bending. The displacement rate was 0.01 mm/s to be consistent with previous testing. The toughness was interpreted in terms of the critical stress intensity at maximum load. This approach is, in principle, the same as that advocated by Ritchie et al. for the testing of small animal bones (Ritchie et al., 2008); it has been here applied

[5] Variations in sample thickness resulted from the size of the cortex thickness in various donors. Individual sample thicknesses varied from 1.4 to 2.0 mm in the Young group, from 1.2 to 1.5 mm in the Aged group, and from 1.3 to 2.4 mm in the Aged Diseased group. Thickness variations did not appear to affect the measured toughness values; indeed Norman et al. report no effect of thickness on bone toughness in the 2–3 mm range (Norman et al., 1995).

to human cortical bone to measure the toughness of whole bone.

To determine R-curves in the transverse orientation, the small humeri and tibiae samples were loaded in three-point bending in accordance with ASTM E1820-08 (2008); tests were performed in 25 °C HBSS at a displacement rate of 0.01 mm/s on an EnduraTec Elf 3200 testing machine (BOSE, Eden Prairie, MN). It has been shown previously that the cracks propagating in the transverse orientation undergo in-plane deflection and through-thickness twisting creating a mixed-mode driving force for crack extension (Koester et al., 2008). Additionally, as there are significant inelastic (plasticity) mechanisms in bone which contribute to its intrinsic toughness, we used here a nonlinear elastic fracture mechanics approach (Koester et al., 2008; Ritchie et al., 2008; Yan et al., 2007) and calculated the crack-driving force using the *J*-integral, where *J* is the nonlinear strain-energy release rate defined as the rate of change in potential energy in a nonlinear elastic solid for unit increase in crack area (Rice, 1968). Crack lengths were estimated in terms of the equivalent through-thickness crack of the same compliance.[6] Specifically, to monitor crack extension, measurements of the elastic compliance, C_{LL}, were made during periodic unloading (~10%) every ~25 μm of crack extension during the R-curve test. The relationship between C_{LL} and the equivalent through-thickness crack length, *a*, was obtained from handbook solutions. Resulting toughness values at each measured crack length were measured in terms of the sum of the elastic, J_{el}, and plastic contributions, J_{pl}, to *J* (ASTM E1820-08, 2008):

$$J = J_{el} + J_{pl}. \tag{1}$$

The elastic component was determined from:

$$J_{el} = K^2/E \tag{2}$$

where *K* is the stress intensity and *E* is Young's modulus. This contribution was quite small, and typically only 5%–10% of J_{pl}. The plastic component of *J* was calculated from:

$$J_{pl} = \frac{2A_{pl}}{Bb} \tag{3}$$

where A_{pl} is the area under the plastic region of the load *vs.* load-point displacement curve, *B* is the specimen thickness, and *b* is the (macroscopic) uncracked ligament (*W*–*a*).

The validity of these *J*-based toughness measurements is achieved provided *J*-dominance is maintained at the crack tip, *i.e.*, that the value of *J* fully characterizes the crack-tip stress and displacement fields. This is achieved when the uncracked ligament *b* exceeds 10 J/σ_y where σ_y is the flow stress; additionally, plane strain conditions prevail where the thickness $B > b$ (ASTM E1820-08, 2008). Taking $\sigma_y \sim$ 100 MPa, this implies that both *J*-dominance exists for the crack-initiation toughnesses when *b* exceeds 0.2 mm, which is readily met in all our test samples. Indeed, even the crack-growth toughnesses up to ~20 kPa m still meet these validity criteria.

As it is somewhat uncommon to express the toughness of biological materials, such as bone, in terms of *J*, the toughness results in this paper were additionally expressed in terms of the stress intensity; specifically equivalent (effective) stress intensities were computed from the standard *J*–*K* equivalence (mode I) relationship (ASTM E1820-08, 2008):

$$K_J = \sqrt{JE}. \tag{4}$$

The back-calculation of equivalent *K*-based toughness values requires knowledge of the Young's modulus *E*. To determine whether the value of *E* was affected by aging, measurements *of the elastic stiffness* were made on *Young* and *Aged* cortical bone using nanoindentation. A total of 15 indentations was performed on each of the two groups with a Triboindenter (Hysitron, Inc., Minneapolis, MN). At each location, the reduced modulus of the bone tissue material was determined. Results showed that the *Young* and *Aged* bone had a reduced elastic modulus of 16.99 (±3.8) GPa and 17.11 (±3.5) GPa, respectively, giving a true elastic modulus of $E = 15.70$ (±3.5) GPa for *Young* bone and 15.85 (±3.3) GPa for *Aged* bone. These values were used with Eq. (4) to compute the equivalent *K* toughness values. The observed lack of an effect of aging on the elastic modulus of bone is consistent with previous studies in the literature (Wang et al., 2002; Hoffler et al., 2000).

To observe mechanisms, specifically the nature of the crack path in relation to the bone microstructure, three-point bend tests were also conducted on hydrated *Young* and *Aged* bone *in situ* in a Hitachi S-4300SE/N ESEM environmental scanning electron microscope (Hitachi America, Pleasanton, CA) using a GatanMicrotest 2 kN three-point bending stage (Gatan, Abington, UK). This technique (Koester et al., 2008) further permitted measurements of the R-curve while simultaneously imaging the crack path in back-scattered electron mode at 15 kV and a pressure of 35 Pa.

Additionally, synchrotron X-ray computed micro-tomography was performed on two specimens each of the *Young* and *Aged* groups at the Advanced Light Source (beamline 8.3.2), at the Lawrence Berkeley National Laboratory; the setup is similar to standard tomography procedures (Kinney and Nichols, 1992) in that samples are rotated in a monochromatic X-ray beam and the transmitted X-rays imaged via a scintillator, magnifying lens and a digital camera to give an effective voxel size in the reconstructed three-dimensional image of 1.8 μm. Samples were scanned in absorption mode and the reconstructed images were obtained using a filtered back-projection algorithm. In absorption mode, the gray-scale values of the reconstructed image are representative of the absorption coefficient. To maximize the signal-to-noise ratio, an input X-ray energy of 20 keV was selected; this optimizes the interaction between the X-rays and the sample. Two-dimensional images were taken every quarter of a degree between 0° and 180°. The

[6] Although we regularly use optical measurements of crack length to verify those deduced from compliance measurements, in the present study focused on fracture in the transverse orientation, the crack is subject to marked deflections and tortuosity, both in-plane and through-thickness (see Fig. 4), such that it becomes increasing difficult to define exactly what the crack length is. In light of this, the fracture mechanics procedure of using compliance measurements to discern the length of the equivalent through-thickness crack with the same compliance was employed.

data sets were then reconstructed using the software Octopus (Vlassenbroeck et al., 2007) and the three-dimensional visualization was performed using AvizoTM software (Mercury, 2008).

Whereas the ESEM gives high-resolution two-dimensional images of the crack/microstructure interactions, X-ray computed micro-tomography provides slightly lower-resolution three-dimensional imaging of the nature of the crack trajectories in relation to the osteonal structure within the cortical bone.

3. Results

The results of the crack-growth resistance-curve measurements (K_J and J as a function of crack extension Δa) for the various groups of cortical bone in the transverse orientation are presented, respectively, in Fig. 3(a) and (b); as there is considerable variation in the individual R-curves, scatter bands for these groups are plotted in Fig. 3(c) and (d) to highlight the trends in these data. It is apparent from these figures that in general both the crack-initiation toughness (at $\Delta a \rightarrow 0$) and the crack-growth toughness (slope of the R-curve) are decreased with age, although compared to published results for the longitudinal orientation (Fig. 2) the effect is quite small. The age-related decrease in crack-initiation toughness in this orientation appears to be on the order of ~6%, far smaller than the factor of ~2 reported (Nalla et al., 2004) for the longitudinal orientation over a similar age range. The decrease in crack-growth toughness was larger, on the order of ~14%, but this again is far smaller than the almost order of magnitude decrease reported (Nalla et al., 2004) for the longitudinal orientation. Actual values are listed in Table 1. Toughness results are actually not that different between the healthy young and aged groups although a more significant effect is seen for bone from the donor with diabetes, where although the average decrease in the growth toughness was ~14%, *i.e.*, similar to that for aged healthy bone, the decrease in the initiation toughness was 40%, all as compared to the toughness values for Young bone.

Corresponding whole bone fracture toughness measurements on the human femurs were analyzed using the maximum load procedure (Ritchie et al., 2008). However, due to the scarcity of large sections of human femurs suitable for this type of analysis, it was only possible to analyze two femurs. It was found that the 'whole bone' toughness in the transverse direction for the femur from a 48-year-old male was 6.67 MPa$\sqrt{\text{m}}$ and the toughness of femur from a 79-year-old male was 6.13 MPa$\sqrt{\text{m}}$.

In situ crack growth in an environmental scanning electron microscope has been used successfully previously (Koester et al., 2008; Launey et al., 2010) to study the extrinsic toughening mechanisms that confer toughness in bone. In the present study, cracks were grown in the transverse direction in *Young* and *Aged* bone samples specifically to compare the active extrinsic toughening mechanisms in this orientation. Results, shown in Fig. 4 based on both *in situ* ESEM and computed X-ray micro-tomographic imaging, indicate extensive crack path meandering which is characteristic of bone fracture in the transverse orientation. On initiating from the sharpened notch, cracks can be seen to deviate away from the path of maximum tensile stress (which is co-planar with the notch plane) and to undergo marked deflections, of 90° or so (in three dimensions, crack twisting is evident too), on encountering the cement lines of the osteons. Mechanistically, such crack deflection/twist, which is the principal source of microscopic toughening for the transverse orientation (Koester et al., 2008), was similar in both *Young* and *Aged* samples; however, whereas the number of deflections was often increased in the older bone, the deflections were smaller in magnitude and the resulting overall crack paths were less tortuous (Fig. 4).

4. Discussion

Resistance to fracture in bone results from a suite of physical structure-related mechanisms that act at multiple length-scales ranging from nano- to near macro-scale dimensions (Fig. 5) (Launey et al., 2010). As noted above, these mechanisms can be classified in terms of their intrinsic and extrinsic contributions to bone toughness. At smaller size-scales, toughening in bone arises primarily from "plasticity" mechanisms that operate principally at sub-micrometer dimensions to promote intrinsic toughness. These mechanisms, which include molecular uncoiling of collagen molecules, fibrillar sliding of both mineralized collagen fibrils and individual collagen fibers, and microcracking, toughen primarily by forming "plastic" zones surrounding any growing cracks (Launey et al., 2010; Fratzl et al., 2004; Fantner et al., 2005; Buehler, 2006). Such intrinsic mechanisms tend to principally affect the crack-*initiation* toughness. At coarser size-scales, toughening in bone is primarily associated with extrinsic crack-tip shielding mechanisms that operate at length-scales of ~1–100 s μm to promote the crack-*growth* toughness, with the salient processes being crack deflection/twist and crack bridging (Nalla et al., 2005; Launey et al., 2010; Nalla et al., 2003; Koester et al., 2008). A central feature of the latter toughening mechanisms is the specific nature of the crack path which is controlled by the applied forces and the nature of the bone-matrix microstructure, in particular the hyper-mineralized interfaces of the osteons (cement lines), which provide microstructurally 'weak', and hence preferred, paths for cracking. Microcracks most often form at these cement lines; they are thus primarily aligned along the long axis of the bone with a typical spacing of ~10–100 s μm (Koester et al., 2008). Wagner and Weiner (1992) and Weiner et al. (1999) have shown that the mineral crystals are plate-like along the collagen, forming a plywood structures with an alternating angle between different lamellae; the local change in orientation around the cylindrical osteons from the *c*-axis of the mineral is one reason that the crack deflects along the cement lines and circumvents the osteons (Wagermaier et al., 2007). Indeed, a recent study has shown that 99% of all cracks in bone are aligned at an angle of less than 25° with respect to the osteons (Wasserman et al., 2008). It is because of the orientation dependence of the microcracking, and the arrest and crack deflection as the crack encounters the cement lines

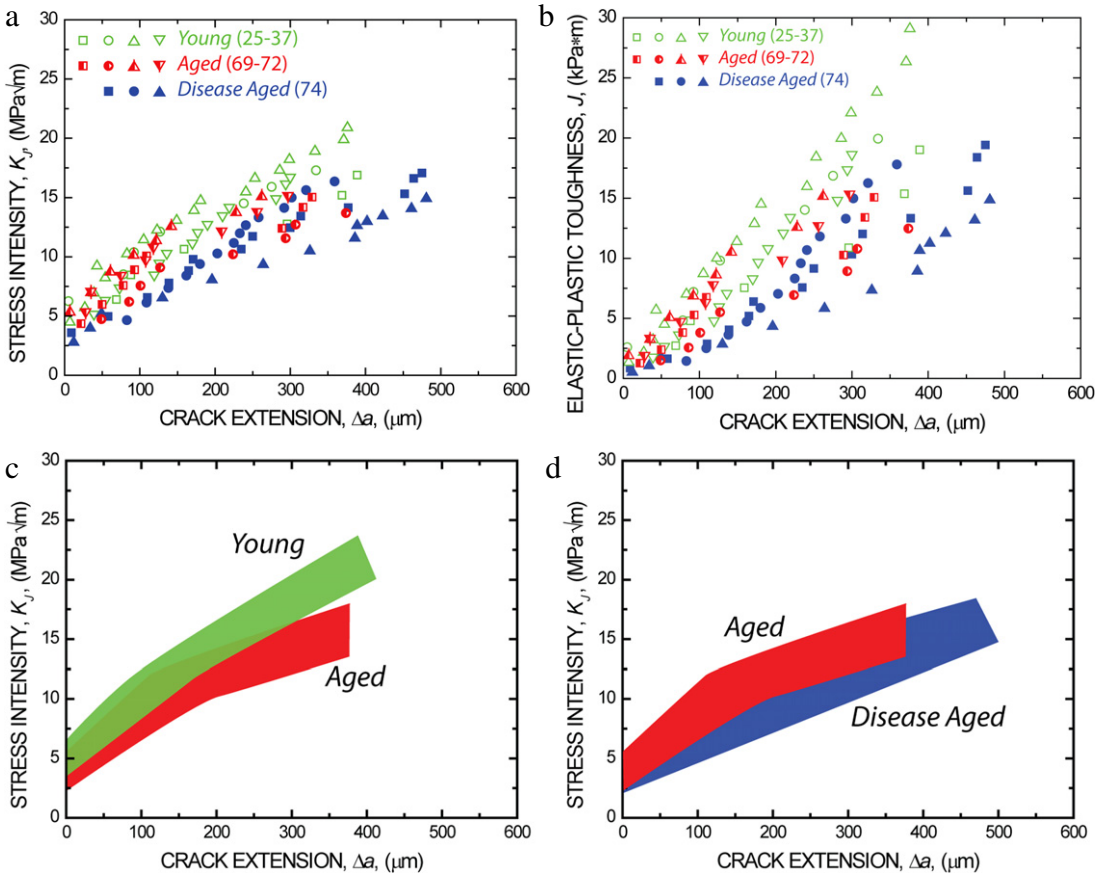

Fig. 3 – Effect of aging on the fracture toughness of human cortical bone in the transverse orientation, showing (a) $K_J(\Delta a)$ and (b) $J(\Delta a)$ resistance curves for stable *ex vivo* (25°C HBSS) crack extension in human bone in three groups: *Young* (25–37 years), *Aged* (61–69 years) and Disease Aged (74 years). For the comparison of *Young vs. Aged*, note the small average decrease (~6%) in crack-initiation toughness (defined at $\Delta a \rightarrow 0$) compared to the larger (~14%) decrease in crack-growth toughness (slope of the R-curve) with aging. Samples were orientated with the starter notch and the nominal crack-growth direction along the transverse direction of the cortical bone, *i.e.*, perpendicular to the long axis of the osteons. Note that all toughness measurements were made in terms of J using nonlinear elastic fracture mechanics methods; equivalent K_J values were back-calculated from these J values, as described in the text.

(Koester et al., 2008; Peterlik et al., 2006), that bone is much more difficult to *break* than to *split*.

In the longitudinal orientation, (cement-line) microcracks are aligned roughly parallel to the growing crack; their formation alongside and ahead of the crack tip leaves locally intact regions that can act as bridges across the crack, termed "uncracked-ligament" bridging, and can carry load that would otherwise be used to promote cracking (Nalla et al., 2003). The extent of toughening, however, is far larger in the transverse direction as the microcracks are now aligned roughly perpendicular to the crack path where they act as "delamination barriers" (Koester et al., 2008; Peterlik et al., 2006); this serves to locally arrest growing cracks, cause marked crack deflections and crack twists, generate highly tortuous crack paths, extremely rough fracture surfaces, and correspondingly high toughness.[7]

Both the intrinsic and extrinsic toughening mechanisms are known to degrade with aging (*e.g.* Nalla et al., 2004, 2006). As noted above, R-curve measurements in the *longitudinal* orientation of human cortical bone have revealed reduced contributions to the crack-initiation and growth toughness with aging. Specifically, for bone aged between 34 and 99 years, the initiation toughness has been shown to be reduced by a factor of ~2, whereas the growth toughness decreased by over an order of magnitude (Fig. 2) (Nalla et al., 2004). Mechanistically, such an aging-related deterioration in (i) the initiation toughness can be traced, at least in part, to increased cross-linking of the collagen (detected in Raman studies) (Nalla et al., 2006), which suppresses plasticity in bone (from mechanisms such as fibrillar sliding), and

[7] This follows because of the reduced local stress field due to crack blunting and the need to reinitiate the crack following local arrest at such delaminations; in addition, such gross crack-path deviations away from the plane of maximum tensile stress greatly diminish the local stress intensity at the crack tip, thereby necessitating higher applied loads to continue cracking (Nalla et al., 2005).

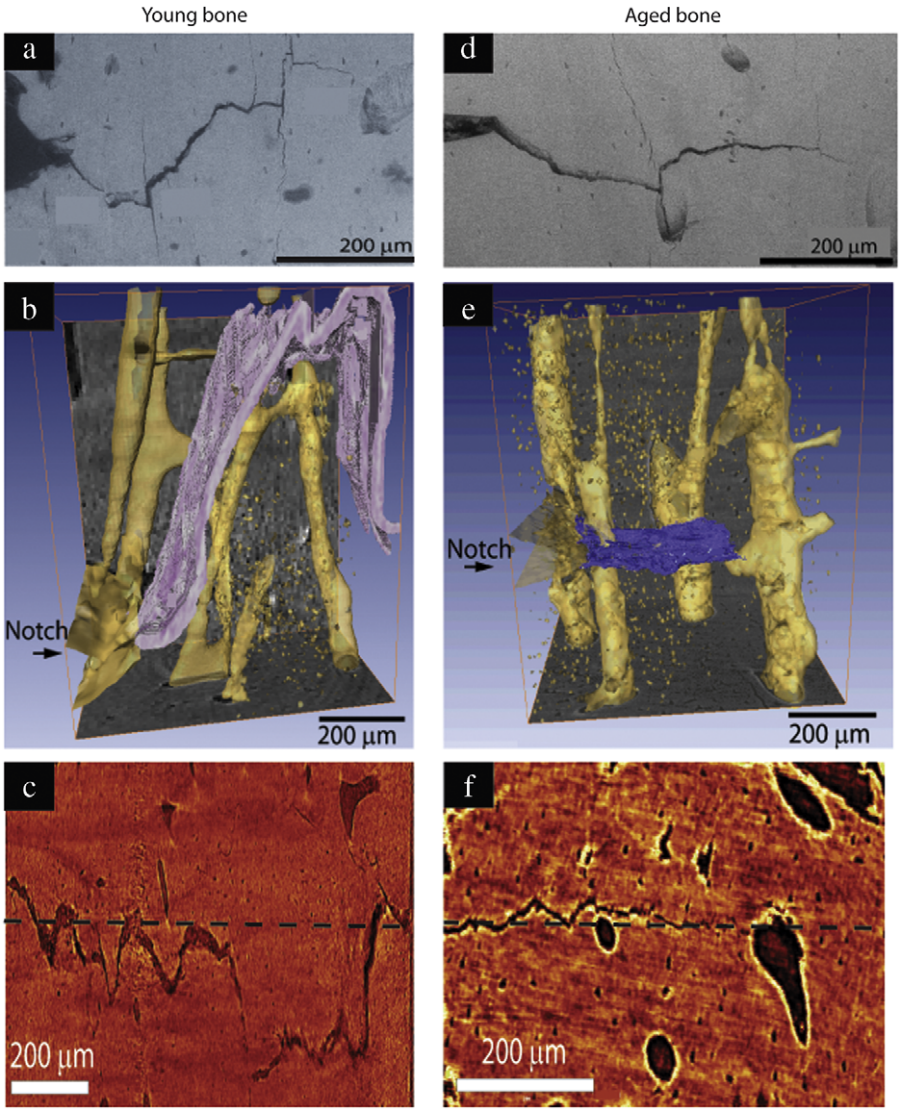

Fig. 4 – Scanning electron microscopy and synchrotron X-ray micro-tomography of crack paths in (a)–(c) *Young* and (d)–(f) *Aged* human cortical bone in the transverse orientation. Images show crack paths: (a) & (d) SEM micrographs from side-view perpendicular to the crack plane, (b) & (e) 3D X-ray micro-tomography images of these paths; 3D crack surface is purple; Haversian canals are yellow brown, and (c) & (f) 2D tomographs of the paths from the back face of the sample. The crack deflects on encountering the osteons; such crack deflection and crack twisting is the prime extrinsic toughening mechanism in bone in the transverse orientation. Note, however, that the frequency of such deflections is increased whereas their severity is decreased with aging, resulting in less meandering crack paths in aged bone.

(ii) the growth toughness from the diminished effect of crack bridging (the primary microscopic toughening mechanism in this orientation) due to the higher osteonal density in older bone (Nalla et al., 2004, 2006). The relationship between osteonal density and potency of crack bridging follows because these bridges are created through the formation of (cement-line) microcracks ahead of a growing crack tip, their average size being a function of the spacing of the osteons. As the density of osteons increases with aging due to Haversian remodeling, the correspondingly smaller spacing of the cement lines results in smaller crack bridges, with a compromised capacity to toughen the bone; this is the reason for the markedly reduced crack-growth toughness (in the form of a much lower slope of the R-curve) with aging (Nalla et al., 2006).

The current results for human cortical bone reveal a somewhat different story for the transverse orientation. Although the bone toughness is again degraded by aging, the effect is far smaller. Over the age range of 25–72 years (for non-diabetic bone), the initiation and growth toughnesses are reduced, respectively, by ~6% and 14%, as compared to corresponding ~15% and 62% reductions in the longitudinal orientation. This is not to say that the effects of aging on the factors that fracture bone are smaller than first thought, as most actual (*in vivo*) bone fractures occur under mixed-mode conditions, *i.e.*, due to the bone geometry and actual physiological loading, they often involve combinations of

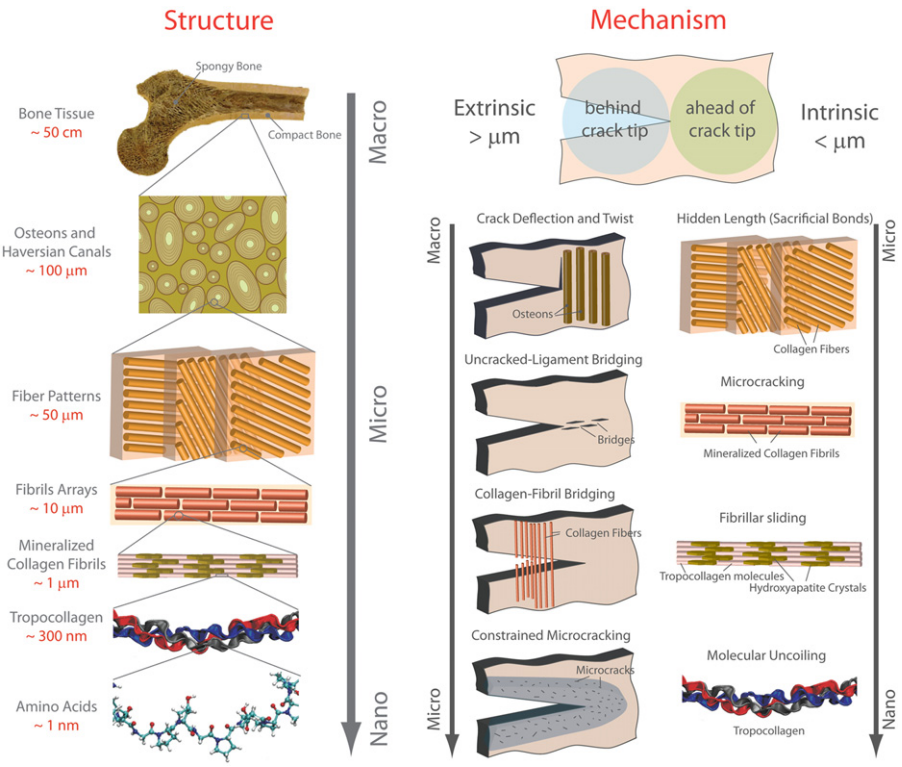

Fig. 5 – The structure of bone showing the seven levels of hierarchy with the prevailing toughening mechanisms. At the smallest level, at the scale of the tropocollagen molecules and mineralized collagen fibrils, (intrinsic) toughening, *i.e.*, plasticity, is achieved via the mechanisms of molecular uncoiling and intermolecular sliding of molecules. At coarser levels at the scale of the fibril arrays, microcracking and fibrillar sliding act as plasticity mechanisms and contribute to the intrinsic toughness. At micrometer dimensions, the breaking of sacrificial bonds at the interfaces of fibril arrays contributes to increased energy dissipation, together with crack bridging by collagen fibrils. At the largest length-scales in the range of 10 s–100 s µm, the primary sources of toughening are extrinsic and result from extensive crack deflection and crack bridging by uncracked ligaments, both mechanisms that are motivated by the occurrence of microcracking.
***Source:* Adapted from Launey et al. (2010).**

tensile, compression and shear forces that result in highly complex fractures along both the transverse and longitudinal directions. However, it is of note that mechanistically the nano/microstructural features associated with the aging of human cortical bone have a far greater influence on splitting rather than transverse (breaking) fractures.

Results from the notched three-point bending tests on whole bone were consistent with this conclusion. Although only a few tests could be performed, they revealed only a modest decrease (~8%) in the transverse toughness with aging. While it would be inappropriate to draw too much conclusion from such limited whole bone tests, the combined R-curve and whole bone results in this study clearly indicate only a marginal reduction in the bone toughness in the transverse orientation with aging.

It is reasoned that the effect of aging on the *intrinsic* contributions to the toughness would not be expected to be that sensitive to bone orientation; in terms of bone fracture resistance, these are primarily associated with the suppression of the "plasticity" in bone from fibrillar sliding due to increased collagen cross-linking. If this were the major contribution to toughness, we would expect this aging effect to degrade the initiation toughness in any orientation, which is clearly not the case. Conversely, it appears that it is the extrinsic contributions to the toughness from the differing crack paths in the longitudinal and transverse directions that play the discerning role. As noted above, aging leads to an increase in osteonal density, which results in a reduced spacing between the cement lines. Since the cement lines are the prime location for microcracking, in orientations where crack bridging is the principal source of microscopic toughening, *i.e.*, the longitudinal orientation, the consequent formation of smaller bridges results in a reduced toughness, which we have concluded is the root cause of the aging-related loss in R-curve toughness in this orientation.

For the transverse orientation, the reduced osteonal spacing should result in more frequent crack deflections as the growing crack encounters the cement lines, and this is what is observed (Fig. 4). At first sight, this might be expected to increase the toughness, but the result of these more frequent crack deflections is that the extent of the individual "delaminations" along the cement lines (nominally perpendicular to the main crack path) becomes smaller so that the overall degree of crack-path meandering is actually lessened (*c.f.*, Fig. 4(c) and (f)). A similar effect has been seen in severely irradiated bone (Barth et al., 2010). Thus, the aging-related reduction osteonal spacing leads to shorter crack-path

excursions away from the plane of maximum tensile stress and the overall decrease in crack-path tortuosity, which in turn results in a smaller influence of aging on the fracture toughness in this transverse orientation.

Although only limited tests were performed on diabetic and aged bone, our preliminary results do suggest that the degradation effect of this disease on the bone toughness is quite different from the effect of pure aging. Specifically, compared to non-diabetic bone of a similar age, the crack-growth toughness was not that different; however, the crack-initiation toughness was significantly lower by more than 30%. This implies that diabetes does not necessarily affect the larger-scale microstructure of bone that gives rise to extrinsic toughening mechanisms, *i.e.*, bone-matrix structure at micrometer length-scales and above (in particular the secondary osteons), but rather the intrinsic ("plasticity") mechanisms that are principally affected by structure at sub-micrometer dimensions (Fig. 5). Mechanistically, this clearly is a topic worth pursuing to achieve an improved understanding of the effect of different diseases on the mechanical properties of bone. However, the current work clearly shows the importance of R-curve toughness in such studies as these measurements enable the distinction between effects on the initiation toughness, which are controlled principally by sub-micrometer structure, and on the growth toughness, which are controlled by structure at much larger dimensions (up to the hundreds of micrometers).

Finally, it should be noted that as most actual bone fractures in humans are rough (multi-orientated) mixed-mode failures, our current observations that the transverse fracture toughness of human cortical bone is only slightly affected by aging is still consistent with the notion of an increased risk of fracture with age due to lower bone quality. Although in medical terms this problem is currently treated solely in terms of the aging-related loss in bone mass, the fracture toughness R-curves shown in Figs. 2 and 3 of this paper clearly highlight a concurrent and significant loss in the bone's resistance to fracture. Hopefully future therapies can be developed to treat this aspect of the problem, *i.e.*, that of bone quality, as well as the age-related loss in bone quantity.

5. Conclusions

Based on a fracture mechanics study of the effect of aging (25–74 years) on the fracture toughness of human cortical bone in the transverse (breaking) orientation, the following conclusions can be made:

1. R-curve measurements in 25 °C HBSS show the transverse toughness to be only modestly affected by aging. Over the age range of 25–72 years, the crack-initiation toughness was decreased by ~6% and the crack-growth toughness by ~14%. Limited notched whole bone fracture tests similarly show only a modest reduction in transverse toughness with age. This is to be compared with previous measurements on the longitudinal fracture toughness where over the same approximate aging range, the crack-initiation toughness was decreased by ~15% and the crack-growth toughness by over 60%.
2. Based on limited experiments, the corresponding effect of aging of the transverse toughness of aged (74-year-old) diabetic cortical bone, however, was found to be more marked. Compared to young bone, although the crack-growth toughness was similarly reduced by ~14%, the crack-initiation toughness was decreased by as much as ~40%.
3. Mechanistically, whereas the aging-related deterioration in the (extrinsic) toughness in the longitudinal orientation has been associated with an increase in the osteonal density which results in a reduced spacing between the cement lines (the prime location for microcracking) which in turn leads to a diminished toughening role of crack bridging, in the transverse orientation where crack deflection/twist is the prime source of toughening, the smaller spacing of the cement lines in older bone can be seen to cause an increase incidence of crack deflection. However, as these deflections are smaller in magnitude, the resulting overall crack paths are generally less tortuous than in young bone. Consequently, in contrast to the longitudinal toughness, the aging-related effect on the transverse toughness is relatively small.

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