Relations Between Tensile Impact Properties and Microstructure of Compact Bone*

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Summary. Standardized human and beef femoral compact bone specimens were tested in tensile impact and the dynamic mechanical properties were determined. The microstructure of 45 beef and 47 human bone specimens were examined histologically to determine if there is a structural basis to account for strength differences in the bone samples. Strong negative correlations were obtained between the maximum stress and the percentage area of secondary osteons in each specimen. For human bone samples, the energy absorption capacity and the modulus of elasticity were also found to have strong negative correlations with the percentage area of secondary osteons present in each specimen. Linear regression equations were obtained describing the impact strength properties in terms of the percentage areas of secondary osteons and cavities in the samples.

Fracture surfaces of the tested bone specimens were examined in a scanning electron microscope. Most surfaces exhibited a fairly rough texture indicating a quasi-cleavage type of failure. Fractographic analysis of bone fracture surface was helpful in understanding the micromechanics of bone fracture.

Key words: Bone — Strength — Tension — Microstructure — Osteon.

Introduction

The importance of tensile loading in the fracture mechanisms of bone has long been recognized (Currey, 1975; Evans, 1957; Olivo et al., 1937). However, while the impact tolerance of whole bones and the dynamic strength of bone specimens have been investigated in modes other than tension, few attempts have been made to determine the tensile impact strength of bone at high loading rates. Saha and Hayes have previously reported on the tensile impact properties of beef and human compact bone (Saha and Hayes, 1974; 1976).

Many researchers have also shown that microstructural characteristics may significantly affect bone mechanical properties (Evans, 1973). In particular, correlations between bone microstructure and static tolerance limits have been reported (Currey, 1959; Evans and Bang, 1966; Sedlin and Hirsch, 1966; Strandh, 1960; Vose, 1962).

Evans (1958) tested human compact bone specimens in static tension and found no significant correlation between ultimate stress and percentage area of osteons in the specimens. Currey (1959) was the first to establish definitely a statistically significant negative correlation between the static tensile strength and percentage area of haversian systems in beef bone specimens. This was confirmed by Evans and Bang (1966; 1967), who found, in addition, a significant positive correlation between tensile strength and percentage area of interstitial lamellae in the break area of the specimen.

Currey (1975) tested the tensile strength of beef bone at strain rates ranging from 0.00013/s to 0.16/s. He found that increasing reconstruction (as measured by the percentage of haversian systems) decreased yield strength, ultimate strength, and modulus of elasticity, while increasing ash content had the opposite effect.

Evans and Riolo (1970) found a positive correlation between the fatigue life of adult human cortical

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bone and the percentage of osteons and osteons plus fragments in the break area. Carter and Hayes (1976), and Carter et al. (1976) shows a highly significant negative correlation between fatigue life and the extent of haversian remodeling after allowing for density (specific gravity of dry bone) differences. Their data thus suggest that haversian remodeling of primary bovine bone reduces resistance to fatigue not only by decreasing bone density, but also by creating an inherently weaker structure.

Evans and Vincentelli (1969), Vincentelli and Evans (1971), and others have studied the relationship between collagen fiber orientation, degree of mineralization, and ultimate strength of compact bone (Olivo et al., 1937; Vose, 1962; Vose et al., 1959) and single osteons (Ascenzi and Bonucci, 1968; Ascenzi et al., 1973).

The purpose of the present investigation was to explore the relationships between tensile impact properties and the microstructural characteristics of the tested specimens.

Materials and Methods

Compact bone specimens of standardized size and shape (Fig. 1) were machined from the femoral cortex of $\cos 1\frac{1}{2}$ to 2 years old and from the right femur of a 38-year-old human male who died from a brain tumor. The specimens were tested in a pendulum type tensile impact tester (Fig. 2) at a strain rate of 133/s. The load was monitored by a quartz load cell (Kistler model 510, Redmond, Washington) whose output after amplification was recorded on a storage oscilloscope (Tektronix model 564B, Beaverton, Oreg.) as a load-time curve. Due to the constant velocity of impact, the load-time curves also represented the dynamic stress-strain behavior of the specimens, and these were used to calculate mechanical properties.

After mechanical testing, fracture surfaces of selected specimens

were defatted in ethyl alcohol, coated with a 60/40 gold-palladium coating and examined in an AMR 500 scanning electron microscope (Advanced Metals Research Corp., Bedford, Mass.). A suitable metallographic preparation technique was also developed in order to observe the bone specimen microstructure with a reflecting optical microscope. The oblique fracture surfaces of the cortical bone samples were ground manually on fine silicon grit paper to obtain a flat transverse surface as close to the fracture as possible. The samples were then cast in a quick hardening cold metallurgic embedding material (Quickmount, Fulton Metallurgical Products, Pittsburgh, Pa.). As a large number of samples were examined, a special jig was used to cast 8 samples in a single mold.

The embedded specimens were then polished on rotating metallurgic wheels using: (1) 400 and 600 grit silicon carbide paper discs with water as a lubricant and coolant, (2) 15μ diamond abrasive on paper with kerosene as lubricant, and (3) 6μ diamond abrasive on cloth wheels with diamond lubricant. For final polishing we used #2 (0.3μ) and #3 (0.05μ) alumina slurry on sylvyt cloth wheels. Bone microstructure was photographed from the polished samples in a Leitz reflected light microscope at a $50 \times$ magnification (Fig. 3a).

The method of measuring the percentage of cross-sectional area occupied by secondary osteons in each specimen consisted of tracing the outlines of the secondary osteons and joining the osteon outlines by straight lines. The total area of all the osteons was measured by moving the planimeter needle in the same direction around the osteon outlines (Fig. 3b). In this process, the needle traversed the lines joining the osteons twice, and thus did not contribute to the area. The reproducibility of measurements by this method was $\pm 3\%$.

For correlating bone strength with microstructure, only two histologic bone types were considered: (1) haversian systems (secondary osteons) and (2) non-haversian systems consisting of many types of primary bone. Primary osteons were defined as those produced at a surface, de novo, with no other type of bone tissue having existed there previously. Secondary osteons, on the other hand, were formed following the resorption of previous bone of any histologic type. To distinguish between primary and secondary osteons while measuring the area of secondary osteons in each specimen, the following guidelines were used:

1. Secondary osteons are nearly always bounded by a cement line (Fig. 3a).

2. Because secondary osteons are formed subsequent to the bone, they replace the bone matrix surrounding each haversian



Fig. 1. A standardized compact bone specimen for tensile impact test with a strain gage attached at the center to measure strain. The specimens were 57 mm long and 1.6 mm thick



Fig. 2. Test set-up for tensile impact testing



Fig. 3. (a) Photomicrograph of the microstructure of a longitudinal tensile impact specimen of beef bone showing secondary osteons and a resorption cavity (*arrow*), \times 42.5. (b) Measurement of the area of secondary osteons. The movement of the planimeter needle is shown by *arrows*

system which stops abruptly at the cement lines (Fig. 3a). In primary osteons, the surrounding structures follow the osteon curvature much like the streamlines around a smooth body, and often there is no clear demarcation line between them (Fig. 4).

3. A secondary osteon often encroaches on adjacent systems (Fig. 4), while this is not seen in primary osteons.

4. Most secondary osteons have a central haversian canal, unless it is being replaced by a newer haversian system.

5. The concentric lamellar structure of secondary osteons is often more prominent than that of the primary osteons.

Even with these distinguishing factors, in many cases it was difficult to isolate the secondary osteons from the surrounding bone matrix and from the other primary osteons, a difficulty also pointed out by Hert et al. (1965).

In the calculation of the percentage area of secondary osteons, large resorption cavities with newly formed concentric lamellae were considered secondary osteons. The secondary osteonal area also included fragments of older secondary osteons parts of which had been replaced by a new generation of haversian systems. In the beef compact bone samples with highly convoluted microstructure (Fig. 5), large irregular bone masses (marked A in Fig. 5) were not considered as secondary osteons. The osteons in all the beef and human bone samples were measured by the same person without reference to the results of mechanical testing.



Fig. 4. Typical micrograph of a fresh human compact bone specimen. The *arrow* indicates a secondary osteon partially replacing a primary osteon



Fig. 5. Micrograph of a beef bone specimen showing highly convoluted microstructure. The structures similar to that marked by A were not considered secondary osteons

Results

Mechanical Properties and Microstructure. We tested 49 fresh human and 50 bovine bone specimens in tensile impact at a strain rate of 133/s. The tensile impact data and the correlations between strength and elastic properties have been reported elsewhere (Saha, 1973; Saha and Hayes, 1974; 1976). Only the means and standard deviations of the mechanical properties relevant to this discussion are shown in Table 1.

After mechanical testing, photomicrographs of the bone microstructure were taken and the areas of secondary osteons or haversian systems and the cavity areas (spaces occupied by haversian canals, primary canals, resorption cavities, and Volkmann's canals) were measured from the photomicrographs. In calculating cavity area we omitted the canaliculi and lacunae since their combined volume in human cortical bone is less than 3% (Frost, 1963). The mean percentages (\pm one SD) of secondary osteon and cavity areas for 47 human compact bone specimens were 47.5 \pm 13.4 and 12.8 \pm 5.1%, respectively. Evans and Bang (1966) obtained a 45.4% osteon area for 54 human femoral specimens which is in good agreement with our result.

Table 2 shows correlations between mechanical properties and the histologic characteristics for beef and human compact bone. The relationship between the maximum tensile impact strength (σ_u) and the percentage area of secondary osteons in human compact bone has been shown graphically in an earlier paper (Saha and Hayes, 1976). The regression equation between tensile impact strength and area of secondary osteons in human compact bone is

$$\sigma_{\mu} = 212.9 - 1.855x$$
 Eq. 1

where σ_u is given in MN/m² and x is the percentage of

Table 1. Mechanical properties of compact bone specimens in tensile impact (mean ± 1 SD)

Source and condition	Number of samples	Ultimate stress $\sigma_u (MN/m^2)$	Impact energy U(J/m ²)	Maximum strain %	Modulus of elasticity (GN/m ²)	
					Tangent, E	Secant, E_i
Human ^a (fresh) Beef ^b (fresh)	49 50	$\frac{126.3 \pm 33.1}{121.3 \pm 36.5}$	18790 ± 7355 24900 ± 14900	$\frac{1.15 \pm 0.30}{1.34 \pm 0.54}$	14.5 ± 3.4	$12.4 \pm 2.9 \\ 12.6 \pm 2.2$

^a Saha and Hayes (1976) ^b Saha and Hayes (1974)

Table 2.	Correlation	n coef-					
ficients	between	mech-					
anical	properties	and					
microstructure							

	Beef bone	Fresh human bone		
	Area of secondary osteons	Area of secondary osteons (%)	Area of cavity (%)	
Ult. tensile stress (σ_u)	-0.2999^{a}	-0.7352	-0.1136	
	(45) ^b	(47)	(47)	
	S = 0.023 ^c	S = 0.001	S = 0.223	
Impact energy (U)	-0.1707	-0.6962	-0.2311	
	(45)	(47)	(47)	
	S = 0.131	S = 0.001	S = 0.059	
Tan. mod. of elasticity (E)		-0.2707 (47) S = 0.033	-0.0233 (48) S = 0.438	
Ult. strain (ε)	-0.0774	-0.3513	-0.2229	
	(45)	(47)	(47)	
	S = 0.307	S = 0.008	S = 0.066	
Sec. mod. elasticity (E_i)	-0.1832	-0.3209	-0.0520	
	(45)	(47)	(48)	
	S = 0.114	S = 0.014	S = 0.363	
Yield stress (σ_i)		-0.6197 (19) S = 0.002	-0.3716 (19) S = 0.029	

^a Coefficient

^b No. of specimens

° Significance level



Fig. 6. The relation between energy absorption capacity and the percentage area of secondary osteons in the fresh human bone specimens

haversian systems.

Similarly the relationship between the impact energy absorption capacity (U) and the percentage area of secondary osteons in human compact bone is shown graphically in Figure 6. As shown in Figure 6, the linear regression equation describing the impact energy capacity, U, is given by

$$U = 37209 - 384.2x$$
 Eq. 2

where U is given in J/m^2 .



Fig. 7. The relation between the maximum tensile impact stress and the percentage area of secondary osteons in beef bone samples. The specimens with highly convoluted microstructure (Fig. 5) are marked by *squares*

Table 2 also shows that the percentage cavity area did not correlate significantly with the tensile impact properties of human compact bone.

For the 45 beef compact bone specimens, the mean percentage of secondary osteon area was $11.1 \pm 11.0\%$. As shown in Table 2, the maximum tensile impact strength for beef compact bone exhibited a significant negative correlation with the percentage area of secondary osteons. As shown in Figure 7, the regression equation between tensile impact strength and percentage secondary osteon area for beef bone is

$$\sigma_{y} = 135.6 - 1.055x$$
 Eq. 3

where σ_u is in MN/m² and x is the percentage of secondary osteon area.

Table 2 and Figure 8 also show that there was not a statistically significant correlation between impact energy absorption capacity and percentage area of secondary osteons in beef bone specimens. Figure 8 also shows that the beef bone specimens with a highly convoluted microstructure (Fig. 5) did not show any trend of decreased bone strength.

Fractography. To understand better the influence of microstructure on the tensile impact strength of bone, we examined fracture surfaces of selected bone specimens fractographically in a scanning electron microscope. Most compact bone specimens failed without any significant change in their cross section, indicating the brittle fracture behavior of mature bone. Fracture surfaces sometimes contained a natural discontinuity in the bone, such as a blood vessel, showing that the fracture might have originated there. On the



Fig. 8. The relation between the impact energy absorption capacity and the percentage area of secondary osteons in beef bone samples. The specimens with highly convoluted microstructure (Fig. 5) are marked by *squares*

other hand, when an advancing crack met a natural bone cavity, the crack sometimes stopped or deviated, indicating that natural cavities in bone may also serve to contain the progression of the fracture.

Most tensile impact specimens fractured by cleavage causing shearing of both osteons and interstitial bone matrix. Figure 9 shows an example of such a fracture surface for a human compact bone specimen illustrating that human bone specimens did not show signs of osteon pull-out similar to those which occur when fibers pull out in the failure of composites. However, some osteons in beef bone specimens pulled out (Fig. 10a and b) due to weakness of cement line interfaces. Figure 10(b) also shows that the lamella adjacent to the haversian canal fractured in a different manner from the rest of the osteon, as further evidence of the weakness of the interfaces. The paucity of collagen fibers crossing from one osteonal lamella to the next may be the cause of this weakness. Other authors have also described propagation of cracks between lamellae or along the cement lines providing evidence that these interfaces may be weak links in bone microstructure (Piekarski, 1970).

Figure 11 shows a typical example of a tensile impact fracture surface at high magnification. Although the macroscopic appearance of the fracture surfaces varied, the degree of roughness could be related qualitatively to the mechanical properties. At an ultrastructural level, however, most bone specimens showed a characteristic fibrous appearance which might have been due to separation of the mineral crystals from one another and from the organic matrix.



Fig. 10. SEM of tensile impact fracture surface of a beef bone specimen showing partial osteon pull-outs (a) macroscopic view, $\times 43$; (b) magnified view of the area marked by rectangle in (a), $\times 430$



Fig. 9. Scanning electron micrograph (SEM) of a tensile impact fracture surface of a fresh human compact bone specimen. $\times 36.50$

Fig. 11. Fracture surface of a beef bone specimen at a higher magnification showing signs of ductile fracture



 $5 \mu m$

Discussion

In this study, standardized human and bovine compact bone specimens were tested in tensile impact and the mechanical properties related to specimen microstructure and fracture surface topography. A strong negative correlation between mechanical properties in tensile impact and the percentage area of secondary osteons in the human compact bone specimens was found. Similar correlations were reported by Evans and Bang (1967) between static tensile strength and percentage osteon area for embalmed human compact bone specimens.

The beef compact bone specimens exhibited a significant negative correlation between tensile impact strength and the percentage area of secondary osteons. Similar results were reported by Currey (1959; 1975) for beef bone. However, the present study did not demonstrate significant correlations between impact energy absorption capacity and percentage area of secondary osteons for beef bone. Hert et al. (1965) tested beef cortical bone specimens in bending impact and also did not find significant correlations between energy absorption and osteon area.

This lack of correlation for impact energy absorption capacity in beef bone (despite the strong negative correlation for human bone) may be due to the differences in histologic structure of human and bovine bone (Carter et al., 1976). The beef bone specimens were predominantly laminar (circumferential laminae parallel to the endosteal or periosteal surface, Fig. 3a) with secondary osteon areas generally less than 20%. Human specimens were predominantly osteonal, containing more than 20% secondary osteons.

The results of the microstructural analysis of beef bone further demonstrated that specimens with laminar microstructure sustained higher ultimate stress and absorbed more energy to rupture, than did specimens containing secondary osteons. For example, 12 samples with 5% or less osteonal area had a mean tensile impact strength of 153 MN/m^2 and a tensile impact energy of 31,300 J/m². These values were significantly higher than those of all 50 specimens (Table 1).

In horse bone samples of different histologic types, Walmsley (quoted by Currey, 1959) also found a high Young's modulus and a high ultimate stress in bone specimens with circumferential lamella, whereas in bone samples composed of normal haversian patterns, Young's modulus and breaking stress were low. Similar results were reported by Carter et al. (1976) for the resistance of bovine bone to fatigue.

Thus, the results of this investigation are in good agreement with those of previous investigators. Quantitative differences in regression coefficients and in significance levels may well have been due to the use, in this investigation, of fresh human compact bone and dynamic tensile testing. Further reductions in experimental variance could also be expected with the inclusion of bone density as a variable (Carter and Hayes, 1976; Carter et al. 1976).

As pointed out by Currey (1959), there are two possible explanations for these negative correlations between impact strength and the percentage area of haversian bone: (1) that haversian bone has a higher percentage of cavity area and (2) that haversian bone is less mineralized than primary bone. The first effect may not be very important unless the total cavity area is very large. This study demonstrated the lack of correlation between cavity area and bone strength, unless samples with large cavities were also considered. However, there was a significant positive correlation (correlation coefficient = 0.311, S = 0.017) between the area of secondary osteon and area of cavity space. Thus, a higher percentage of secondary osteons means a higher percentage of cavity space, and, consequently, less bone material to resist stress.

The second reason for the reduced strength of secondary osteons is that they are less mineralized than either primary osteons or the surrounding bone matrix. Using X-ray absorption techniques, Amprino and Engstrom (1952) and Amprino (1952) showed that in mammals the calcium content of primary periosteal bone is always higher (5% to 20%) than that of secondary bone. Strandh (1960; 1961) also found the degree of mineralization of highly mineralized haversian systems to be about 3% or 4% lower than that of the interstitial bone. Even though this percentage is small, Vose and Kubala (1959) and Currey (1969) showed that a small percentage change in mineral content may cause appreciable changes in strength.

Another possible reason why specimens with haversian systems are weak is that the interface between secondary osteons and the interstitial bone matrix is weak, facilitating propagation of the crack and leading to failure. Evidence for this was found in the fractographic analysis of the bone fracture surfaces.

The dependence of human bone strength on microstructure suggests several important practical applications. First, such relationships may enable us to predict the impact tolerance of bone. The strong negative correlations between bone strength and percentage osteon area may help explain the reduced fracture tolerance of aging bone which contains a high percentage of haversian systems. Second, using the relationships between elastic modulus and bone microstructure found here, it may be possible to estimate microstructural features such as the percentage osteon area from in vivo ultrasonic measurements.

The correlation between bone strength and microstructure may also have implications in fracture treatment. At present, it is possible to influence the microstructure of healing bone by certain fracture management techniques. When external plaster immobilization is used, bone healing occurs through callus formation, and the healing bone formed is of a woven type. Where internal fixation by compression plating is employed, fracture healing occurs primarily by direct haversian remodeling. The results of the present investigation suggest that the presence of secondary osteons in directly remodeled bone may, in fact, reduce the strength of the healed bone.

References

- Amprino, R.: Rapporto fra procesi di reconstruzione e distribuzione dei minerali nelle ossa I. Richerche esequito metodo studio dell'assorbimento dei raggi roentgen. Z. Zellforsch. 37, 144-183 (1952)
- Amprino, R., Engstrom, A.: Studies on X-ray absorption and diffraction of bone tissue. Acta Anat. 15, 1–22 (1952)
- Ascenzi, A., Bonucci, E.: The compressive properties of single osteons. Anat. Rec. 161, 377–391 (1968)
- Ascenzi, A., Bonucci, E., Simkin, A.: An approach to the mechanical properties of single osteonic lamellae. J. Biomechanics 6, 227-235 (1973)
- Carter, D.R., Hayes, W.C.: Fatigue life of compact bone I. Effects of stress amplitude, temperature, and density. J. Biomechanics 9, 27–34 (1976)
- Carter, D.R., Hayes, W.C., Schurman, D.J.: Fatigue life of compact bone — II. Effects of microstructure and density. J. Biomechanics 9, 211–218 (1976)
- Currey, J.D.: Differences in the tensile strength of bone of different histological type. J. Anat. (Lond). 93, 87-95 (1959)
- Currey, J.D.: The mechanical consequences of variation in the mineral content of bone. J. Biomechanics 2, 1-11 (1969)
- Currey, J.D.: The effects of strain rate, reconstruction and mineral content on some mechanical properties of bone. J. Biomechanics 8, 81-86 (1975)
- Evans, F.G.: Stress and strain in bones. Springfield, Ill.: Charles C. Thomas 1957
- Evans, F.G.: Relations between the microscopic structure and the tensile strength of human bone. Acta Anat. 35, 285-301 (1958)
- Evans, F.G.: Mechanical properties of bone. Springfield, Ill.: Charles C. Thomas 1973
- Evans, F.G., Bang, S.: Physical and histological differences between human fibular and femoral compact bone. In: Studies on the anatomy and function of bone and joints, F. G. Evans, ed., pp. 142-155. Berlin: Springer 1966

- Evans, F.G., Bang, S.: Differences and relationships between the physical properties and the microscopic structure of human femoral, tibial and fibular cortical bone. Am. J. Anat. 120, 79-88 (1967)
- Evans, F.G., Riola, M.L.: Relations between the fatigue life and histology of adult human cortical bone. J. Bone J. Surg. 52-A, 1579-1586 (1970)
- Evans, F.G., Vincentelli, R.: Relation of collagen fibre orientation to some mechanical properties of human cortical bone. J. Biomechanics 2, 63-71 (1969)
- Frost, H.M.: Bone remodelling dynamics. Springfield, Ill.: Charles C. Thomas 1963
- Hěrt, J., Kuchera, P., Vávra, M., Volenik, V.: Comparison of the mechanical properties of both the primary and Haversian bone tissue. Acta Anat. 61, 412–423 (1965)
- Olivo, O.M., Maj. G., Toajari, E.: Sul significato della minuta struttura del tessuto osseo compatto. Boll. Sc. Med. Bologna 109, 369-394 (1937)
- Piekarski, K.: Fracture of bone. J. App. Physics 41, 215-223 (1970)
- Saha, S.: Tensile Impact Properties of Bone and Their Relation to Microstructure. Ph.D. Thesis, Stanford, Calif.: Stanford University 1973
- Saha, S., Hayes, W.C.: Instrumented tensile impact tests of bone. Exp. Mechan. 14, 473-478 (1974)
- Saha, S., Hayes, W.C.: Tensile impact properties of human compact bone. J. Biomechanics 9, 243–251 (1976)
- Sedlin, E.D., Hirsch, C.: Factors affecting the determination of the physical properties of femoral cortical bone. Acta Orthop. Scand. 37, 29–48 (1966)
- Strandh, J.: Microchemical studies on single Haversian systems. II. Methodological considerations with special reference to Ca/P ratio in microscopic bone structures. Exp. Cell. Res. 21, 406 (1960)
- Strandh, J.: Microchemical studies on single Haversian systems. III. Distribution of calcium, phosphorus and nitrogen in microscopic bone tissue structures of varying ages. Biochimica e Biologia Sperimentale I, 60 (1961)
- Vincentelli, R., Evans, F.G.: Relations among mechanical properties, collagen fibers, and calcification in adult human cortical bone. J. Biomechanics 4, 193–201 (1971)
- Vose, G.P.: The relation of microscopic mineralization to intrinsic bone strength. Anat. Rec. 144, 31-36 (1962)
- Vose, G.P., Kubala, A.L.: Bone strength its relationship to Xray determined ash content. Hum. Biol. 31, 262–270 (1959)

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