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Microhardness and Young's modulus in cortical bone exhibiting a wide range of mineral volume fractions, and in a bone analogue

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The relationships between microhardness and mineral content and microhardness and Young's modulus have been determined for cortical bone exhibiting a wide range of mineral volume fractions. These relationships have also been determined for a hydroxyapatite reinforced polyethylene composite which is considered to be an analogue material for bone. Strong non-linear relationships were found between the variables for both materials. For a given volume fraction of mineral the hardness of the natural bone tissue was found to be considerably higher than that of the analogue material. This was attributed to the different ways in which the mineral phase is bound to the matrix in the two materials. The relationship between microhardness and Young's modulus was similar for both materials. The strength of the relationships found suggest that microhardness data is a viable means of estimating the Young's modulus of specimens that do not easily lend themselves to convential testing procedures.

1. Introduction

The hardness of a material is generally defined as its resistance to penetration by an indenter. Although no precise definition of microhardness exists, Buckle [1] suggested that indents of less than 30 μm made with a load of less than 200 g merit the prefix "micro". Hardness testing of metals has for many years proved a very useful non-destructive test and as such has found many applications, for example in quality control [2]. The earliest work on the hardness of bone was by Rössle [3], but Carlström [4] was the first to apply microhardness techniques. Microhardness provides a means of quantifying the physical effects of small scale spatial variation in the composition of bone. Previous studies have utilised microhardness to show: that interstitial bone differs in its properties from secondary osteonal bone [5, 6, 7], that the properties of long bones vary along their length [5, 8], and that younger secondary osteons differ from older ones [7, 9]. Evans [10] showed a weak, though significant, correlation between microhardness and Young's modulus.

The mechanical properties of bone are a consequence of the interaction between the mineral and collagen components. Small variations in the amount of mineral present in a section of bone can have a large influence on its mechanical properties. It has been shown previously that microhardness is correlated with mineral content [4, 5, 8, 11, 12], although the precise nature of the statistical relationship has yet to be established. Lees [13], however, has suggested that not only is the ratio of mineral to collagen an important factor influencing the measurement of microhardness, but that the way in which the two phases are bound to form the microstructure should also be considered. Studies of the relationship between microhardness and mineral content have used bone from a single species and so the magnitude of the observed variables lies within a narrow range. Because of the small variation in the exploratory variable the relationship between it and the dependent variables is not clear. The effect of this may be to mask some of the trends present.

In this study the relationship between microhardness and mineral content in bone from a variety of species has been investigated so that an extended range of mineral contents may be obtained. Identical experiments were conducted on a hydroxyapatite reinforced polyethylene composite in which the mineral content range was similar to the bone specimens used. The composite was considered an analogue for bone in that it consisted of a brittle phase embedded in a plastic matrix. In addition, the hypothesis that microhardness can be used as a test for Young's modulus in small bone specimens has been evaluated.

2. Methods

Material from red deer antler, crocodile nasal bone, Galapagos tortoise femur, blackfoot penguin radius, fin whale tympanic bulla, alligator femur, wallaby femur and tibia and bovine tibia was collected and stored at $-20°$ C until required for testing. The range of mineral volume fractions exhibited by these specimens was found to vary between 0.24 and 0.61. The bone analogue was an injection-moulded composite in which the volume fraction of hydroxyapatite could be varied up to a maximum of 0.5. The preparation and production of this material is described elsewhere [14]. For this study material with mineral volume fractions

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of 0.1, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 were chosen in order to provide a range similar to that of the natural bone tissue.

Two specimens of approximately 1 cm^3 were, whenever possible, removed from each piece of bone. One of each pair was embedded in a polyester resin (Mataserv metallurgical resin) and the polished on various grades of silicon carbide paper down to 1200 grit. The specimens were then polished with a napped cloth impregnated with 6 μm diamond paste. All polishing operations were carried out under constant irrigation with tap water. Final polishing of each specimen was carried out immediately before the hardness test. The polished surface was then blotted with a soft tissue to remove excess water and the specimen placed on the stage of a Schimadzu microhardness tester. For the composite material polishing was limited to the napped cloth with 6 μm diamond paste to remove any surface films. A 50 g load was applied to both the bone and composite specimens as recommended by previous workers [11, 15] for 10 sec using a pyramid-shaped diamond indenter. The diagonals of the indent so formed were then measured at 400 × magnification and the Vickers hardness number calculated using the equation

$$VHN = \frac{1854.4 \times P}{d^2}$$

where P is the applied load in grams and d the mean of the two measured diagonals in micrometers. Each specimen was indented 10 times taking care to observe the recommendations of Amprino [11] on the positioning of each indent. Due to potential edge effects each indent was made two indent diameters away from an edge such as the wall of an Haversian canal or another indent.

The second specimen of each pair was used to calculate the volume fraction of mineral present in each bone, since the original specimen could not be used after being embedded in resin. The volume of each specimen was first calculated using Archimedes' principle before being ashed in a furnace at 800° C for at least 9 h. The volume of mineral was calculated from the ash mass assuming a density of 3.156 mg m^{-3} [16]. The volume fraction of mineral for each specimen was calculated as the mineral volume divided by the original volume.

The small specimens used for microhardness testing were removed from bones that had been used in a previous study of the Young's modulus [17]. The Young's modulus had already been measured in a region of the bone adjacent to that where the hardness value was measured. The pieces of bone available for microhardness testing were of a shape unsuitable for standard testing of Young's modulus and so the values obtained by Currey were assumed to be representative of these test pieces also. The Young's modulus for the hydroxyapatite reinforced polyethylene composite had been previously determined from tensile tests of similar plaques.

3. Results

It has been shown that measurements of the mechanical properties of bone are influenced by the methods of storage and preparation employed [18]. Microhardness is no exception, as demonstrated by Amprino [11] and Weaver [5]. During the course of the present study a number of experiments were performed on specimens of equine bone in order to estimate the influence of preparation and testing procedures on the final results.

TABLE I The effect of embedding the bone specimens in resin on the microhardness

	N	*VHN*	*SD*
Embedded	108	53.5	6.44
Not embedded	108	55.9	7.54

To test the effect of embedding the bone in resin, 18 specimens from a single bone were tested after embedding and 18 specimens from the same bone were tested unembedded. Each specimen was tested six times giving a total of 108 indents in each condition. Table I shows the result of this experiment. Although a t-test shows the means to be statistically different ($t_{214} = 2.5508, p < 0.02$), a 4% difference is unimportant compared with the much larger differences shown by bones with different mineral volume fractions.

Two of the above specimens were tested two months later and then again after a further month to evaluate the effect of freezer storage on the results. Table II gives the means and standard deviations for these measures. A t-test between the values for the bone tested fresh and after three months showed there to be no significant effect due to freezer storage.

In an anelastic material, such as bone, the time over which the deforming load is applied may have an effect. The question also arises as to whether the indent "recovers" following removal of the load since a strain relaxation effect with time has been shown in other techniques of mechanical testing of bone [19]. In an experiment to investigate the effect of time 10 specimens were indented 3 times at each of the following load times; 5, 10, 15, 30 and 45 sec. The results of this experiment are shown graphically in Fig. 1. Although a 5 sec indent period produces a slightly high value for *VHN*, slight variation of loading around 10 sec would appear to have negligible effect on the measured *VHN*. This result suggests that a 10 sec indent is needed to allow a complete deformation to take place.

To investigate any possible recovery of the indent following removal of the load, five specimens were indented 10 times as described in the methods section. Two indents were measured immediately as for the normal testing procedure. Measurement of the remaining 8 indents was delayed for 10, 30 60 and 300 sec. The results of this test are shown in Table III.

TABLE II The effect of prolonged storage on the measured microhardness. The figures shown are the means of 10 indents with the standard deviation of the mean shown in parenthesis

Specimen	Tested fresh	After 2 months	After 3 months
A	53.2	54.4	51.2
	(6.6)	(5.4)	(6.8)
B	52.4	53.2	56.1
	(4.3)	(7.3)	(5.9)

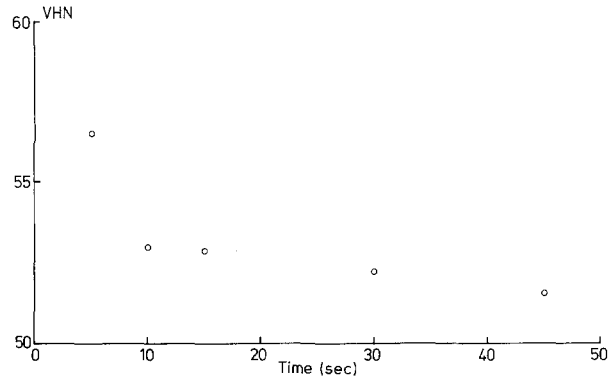

Figure 1 The relationship between microhardness and the time for which the indenter is applied.

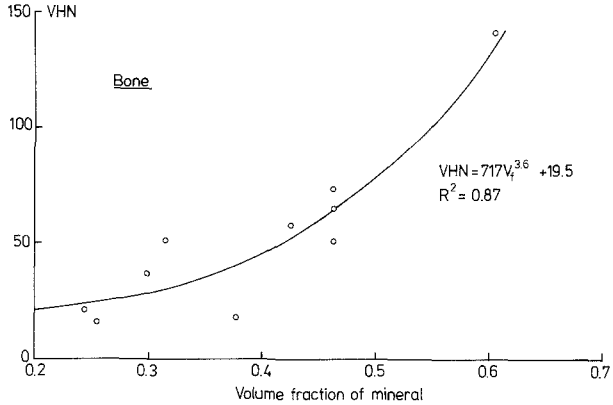

Figure 2 The relationship between microhardness (*VHN*) and mineral volume fraction (V_f) in natural tissue.

These results showed neither pattern nor statistical significance and therefore there appears to be no recovery of the indent following removal of the load.

The indenting load used produced indents varying in diameter between 25 and 75 μm depending on the material. Therefore, the indents could easily be placed within a single osteon or portion of interstitial bone as required. There was no evidence, even in the hardest specimen, of micro-cracking at the corner of the indent. Table IV shows the average indent diameter, the calculated microhardness, the mineral volume fraction and the Young's modulus (calculated from an adjacent specimen), for the natural bone specimens. Table V gives the equivalent data for the hydroxyapatite reinforced polyethylene composite.

Regression analysis was used in order to find a function which gave the best fit of the data for each variable and each material. This was confined to linear variables and power functions with the criterion for the best fit being the regression with the highest coefficient of determination. Table VI summarises the results of this analysis. In all cases the data were extremely well fitted by power functions with a large value obtained for the coefficient of determination (R^2). The table does show, however, that the functions relating the variables are different for the two materials tested.

Figs 2 and 3 show the relationship between microhardness and mineral volume fraction for bone and the composite material respectively. For bone, 87% of the variance, or scatter, in the data is explained by modelling the relationship as a power function with an exponent of 3.6. In the composite the fit is even better with 98% of the variance explained by a cubic relationship. The value of 3.6 for bone shows a relationship very different from the linear one alluded to by other authors, and probably reflects the wider range of the variables assessed in this study. Haque and Turner [20] also found that the volume fraction of hydroxyapatite in a polymer matrix influenced the indentation hardness but only used volume fractions of less than 0.2. The trend was increasing, however, at volume fractions greater than 0.05.

Figs 4 and 5 show the relationship between microhardness and Young's modulus for bone and the composite respectively. For bone, microhardness is related to Young's modulus by a square function, whereas in the composite the relationship is closer to a linear one. In both cases, a very good fit to the data is obtained with 96% and 99% of the variance explained for the bone and composite respectively.

In Fig. 6 the relationship between microhardness and mineral volume fraction is shown for both the natural bone tissue and the composite. This demonstrates more clearly the large differences in *VHN* that exist between the two materials for a given mineral volume fraction. In all cases the hardness of bone is higher than that of the composite, which shows that factors other than the ratio of the two phases present must be influencing the relationship. The same can not be said of the relationship between *VHN* and Young's modulus. Fig. 7 shows that the relationships for the two materials are not dissimilar, and indeed in the region where the two data sets overlap there appears to be no difference.

TABLE III The effect of delaying the measurement of the indent thus allowing for any possible indent recovery (0 sec means immediate measurement). Each figure is a mean of 10 indents

	Time delay before measurement				
	0 sec	10 sec	30 sec	60 sec	300 sec
Mean	53.7	51.0	51.6	55.4	54.6
SD	(5.1)	(5.1)	(3.4)	(4.3)	(6.3)

4. Discussion

This study has established relationships between microhardness and mineral content, and microhardness

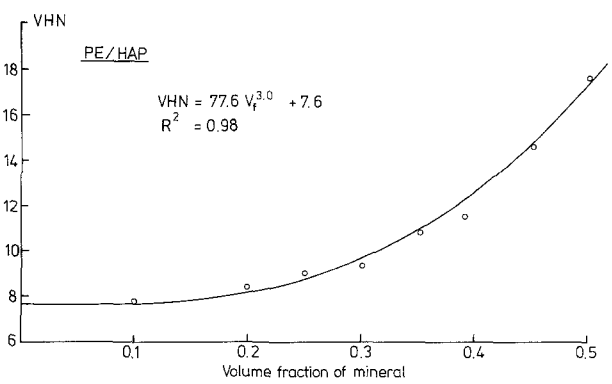

Figure 3 The relationship between microhardness (*VHN*) and mineral volume fraction (V_f) in the hydroxyapatite reinforced polyethlene bone analogue.

TABLE IV The indent diameter, Vickers hardness number (*VHN*), mineral volume fraction and Young's modulus (*E*) of the specimens tested. Values for diameter and *VHN* are means of 10 observations. The Young's modulus data are from [17]

Specimen	Diameter (μm)	*VHN*	Volume fraction	*E* (GPa)
5 year old deer antler	75.5	16.2	0.26	5.5
3 year old deer antler	66.5	21.0	0.25	7.6
Crocodile nasal bone	71.5	18.2	0.38	7.7
Galapagos tortoise femur	49.0	37.6	0.30	11.7
Blackfoot penguin radius	40.0	58.0	0.46	17.0
Wallaby femur	42.5	51.1	0.46	20.0
Alligator femur	42.0	51.5	0.32	14.5
Wallaby tibia	37.5	65.6	0.46	22.8
Bovine tibia	35.0	74.9	0.46	24.5
Whale tympanic bulla	25.5	142.2	0.61	34.1

and Young's modulus in bone for a larger range of mineral contents and moduli than previously shown. Relationships between these variables have also been found for the hydroxyapatite reinforced polyethylene composite. Although the models tested were kept simple, with no attempt made to fit polynominals to the data, the large R^2 values obtained show that they are an adequate way of representing the data. The strength of the relationships found between hardness and modulus are all the more impressive when one considers that the measurements were not taken in the same specimen but from adjacent pieces of material, a factor which in itself would tend to introduce scatter into the data.

The fact that, for a given volume fraction, the hardness of the natural tissue is considerably higher than that of the analogue material is possibly attributable to the different size and distribution of the mineral component and the way in which it is bound to the matrix. The mineral phase of bone is arranged within the matrix in a complex hierarchical arrangement. Marino and Becker [21] first produced evidence from electron paramagnetic resonance that a direct physical bond existed between the collagen and apatite, and evidence from calcified tendon suggests that the apatite crystals are intimately arranged between and within the collagen fibrils [22, 23]. It has been suggested that this bonded arrangement accounts for the tensile stiffness of bone [24], and that it provides a large part of the resistance to indentation [13].

In contrast, Fig. 8 shows that, structurally, the composite material consists of a simple dispersion of approximately spheroidal mineral particles within the polymer matrix. Charalambides [25] tested the same composite in tension and observed flow of the polyethylene around the hyroxyapatite particles and separation between the particles and matrix. This was consistent with a model proposed by Friedrich and Karsch [26] for a hard particulate filler in a ductile matrix with no interfacial bonding. Scanning electron microscopy by Charalambides found no residual polyethylene on the hydroxyapatite particles following fracture. This is further evidence that chemical bonding does not occur between filler and matrix. It would appear, therefore, that the major resistance to the penetration of the indenter comes from a frictional force as the hydroxyapatite particles are forced to move through the matrix under the indenting load, and the resistance of the matrix, rather than from breaking interfacial bonds. Further evidence for this come from Haque and Turner [20] who showed that increasing the crosslinking of the matrix polymer increased the microhardness of a silicate-filled glassy polymer.

It appears, therefore, that it is not only the proportion of filler to matrix that is important in determining the mechanical properties of a composite, but the way in which the two phases are bound also has an effect. The differences in the two materials tested in the present study clearly demonstrate that the presence of a particular volume fraction of mineral in a polymer matrix is not sufficient to explain the microhardness value obtained.

The theory of hardness testing suggests that it is related to the yield strength of the material, rather than the Young's modulus, since the indent is formed as a result of plastic flow in the material. Consideration of the ideal strength of a material, however, shows that strength is theoretically related to the modulus divided by ten [27]. Thus, the correlations observed in this study between modulus and hardness would be expected in an ideal solid. This study has shown that

TABLE V Mineral volume fraction, indent diameter, microhardness (*VHN*) and Young's modulus (*E*) for the hydroxyapatite reinforced polyethylene composite

Volume fraction	Diameter (μm)	*VHN*	*E* (GPa)
0.10	107.9	7.9	1.4
0.20	104.7	8.5	2.0
0.25	101.0	9.1	2.5
0.30	99.6	9.4	3.0
0.35	92.3	10.9	3.7
0.40	89.6	11.6	4.4
0.45	79.3	14.7	5.9
0.50	72.2	17.8	7.7

TABLE VI Equations relating microhardness (*VHN*) to mineral volume fraction (V_f) and Young's modulus (*E*) for bone and the hydroxyapatite reinforced polyethylene composite. R^2 is the coefficient of determination

Bone		Composite	
Equation	R^2	Equaiton	R^2
$VHN = 717(V_f)^{3.6} + 19.5$	0.87	$VHN = 77.6(V_f)^{3.0} + 7.6$	0.98
$VHN = 0.104(E)^{2.0} + 17.5$	0.96	$VHN = 0.64(E)^{1.4} + 6.8$	0.99

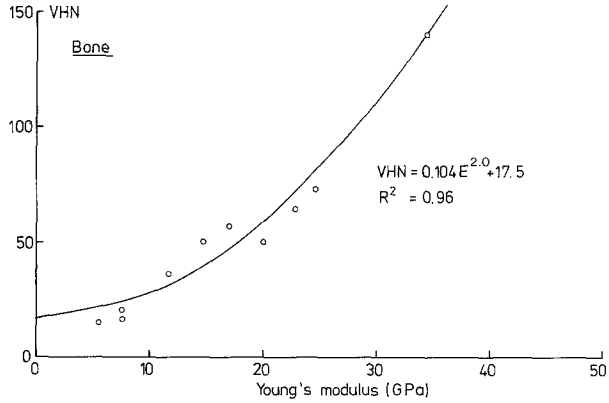

Figure 4 The relationship between microhardness (*VHN*) and Young's modulus (*E*) in natural bone tissue.

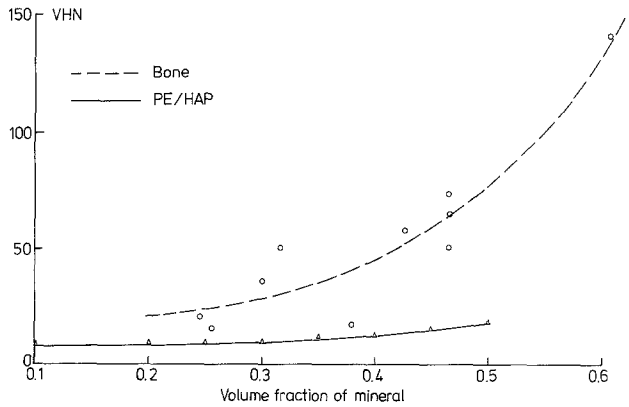

Figure 6 The relationship between microhardness (*VHN*) and Young's modulus (*E*) in the hydroxyapatite reinforced polyethylene bone analogue.

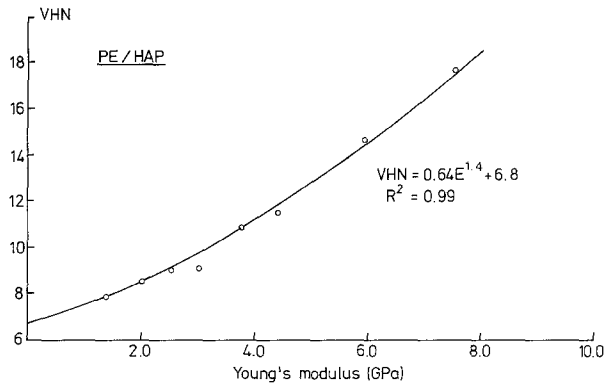

Figure 5 The relationship between microhardness (*VHN*) and Young's modulus (*E*) in the hydroxyapatite reinforced polyethylene bone analogue.

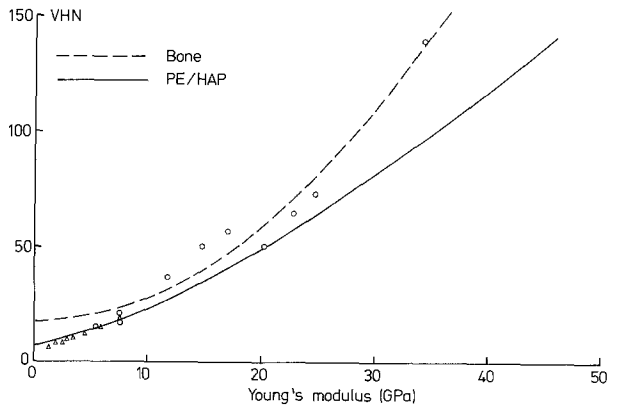

Figure 7 The relationship between microhardness (*VHN*) and Young's modulus (*E*) for both natural bone tissue and the hydroxyapatite reinforced polyethylene bone analogue.

a relationship also holds in solids that are less than ideal.

The strength of the relationships shown in this study suggests that microhardness could be used as an indicator of Young's modulus in bone specimens that do not easily lend themselves to normal testing procedures. These would include specimens such as the subchondral bone of the femoral head [28], or cortical bone near the diaphysis of long bones where the thickness of the bony wall precludes the removal of tensile or three point bend specimen. Additionally, small samples such as those removed through biopsy could be tested, thus giving valuable information about both normal and pathological bone. The non-linear

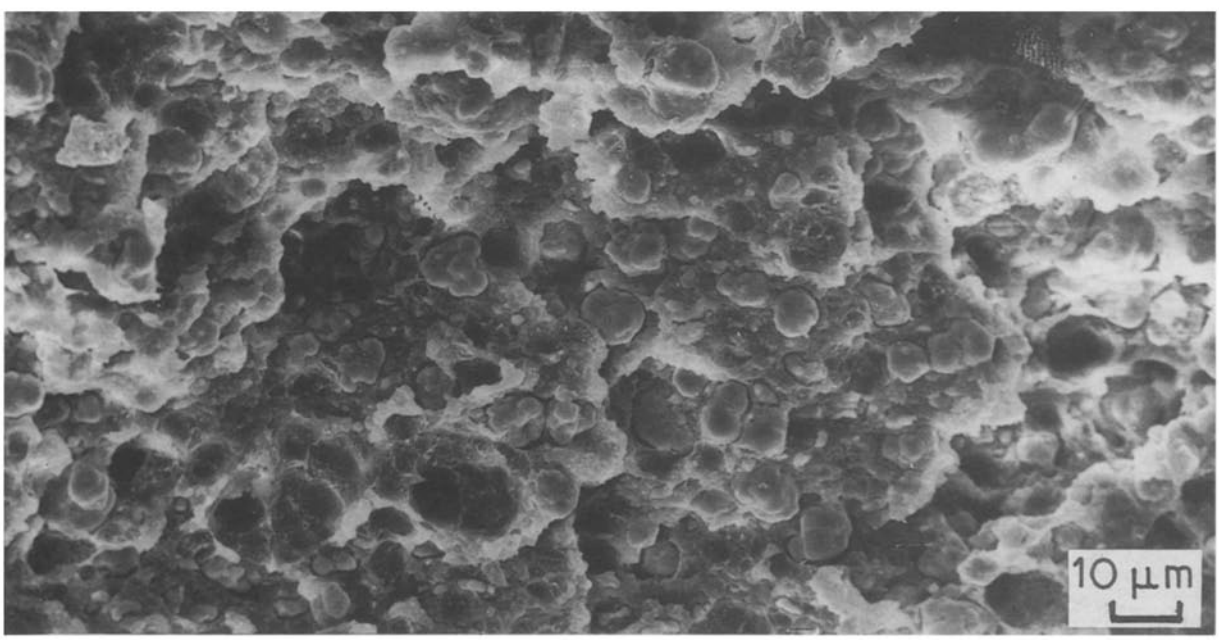

Figure 8 A scanning electron micrograph of a fracture surface in the bone analogue material showing the distribution and size of the hydroxyapatite particles. The volume fraction of this specimen is 0.40.

relationships found in this study, however, illustrate the importance of defining the nature of the relationship between the variables before such an approach is feasible.

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