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Viscoelastic properties of composites of calcium alginate and hydroxyapatite

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Abstract Calcium alginate was reinforced with hydroxyapatite (HA) particles, whose dimensions were a few micrometres, with mass fraction, m_f, values in the range 0–0.8. Cylindrical samples of these composite materials were subjected to cyclic compression in the frequency range $f = 0.001$–20 Hz; in these tests a sinusoidal load of amplitude 1 N was applied either side of a static compression of 2 N. Storage and loss moduli, E' and E'', respectively, were found to be independent of particle size; however, E' increased with frequency consistent with the materials undergoing a glass transition. Above frequencies of about 0.05 Hz, $E' > E''$ for all materials. For each frequency, the dependence of the moduli on $\log_{10} f$ could be represented by a third order polynomial; these equations can be used to calculate E' and E'' for a range of compositions. Approximate values of $|E^*| = \sqrt{E'^2 + E''^2}$ are predicted by a Reuss model.

1 Introduction

This paper presents the viscoelastic properties of calcium alginate hydrogels reinforced by particles of hydroxyapatite (HA). Alginates are polyanions that can be cross-linked by calcium ions to form a rigid hydrogel [1]. These calcium alginate hydrogels are biocompatible and have been used in wound dressings, drug delivery systems, tissue fillers and scaffolds for tissue engineering [2]. Their main disadvantage is that their stiffness is too low for some applications [3]. HA is a biocompatible ceramic [4] that closely resembles bone mineral [5, 6]. It has been used to reinforce polymers in biocompatible composite materials [7–13]. Calcium alginate hydrogels stiffened with HA have been investigated as synthetic bone fillers and tissue engineering scaffolds [3, 9, 13]. However, their viscoelastic properties have not previously been thoroughly investigated.

The properties of calcium alginate/HA composites, as a function of frequency, have now been measured in cyclic compression tests. Properties were measured for a range of HA particle sizes and for a range of composite compositions. Cyclic tests provide values for the storage, E', and loss, E'', moduli that are the real and imaginary parts of the complex modulus, E^* [14]. E' represents the elastic part of the response (in which deformation energy is stored and used in recoil when the stress is removed) and E'' represents the viscous part of the response (in which deformation energy is dissipated and the material flows). It is also conventional to consider values of the real number

$$|E^*| = \sqrt{E'^2 + E''^2} \tag{1}$$

and

$$\tan\delta = E''/E'. \tag{2}$$

When $\tan\delta = 0$, the material behaves as an elastic solid (using all the deformation energy in recoil); when $\tan\delta = 1$, the material behaves as a liquid (dissipating all its deformation energy in flow and not recoiling). A viscoelastic material has $0 < \tan\delta < 1$.

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2 Materials and methods

2.1 Materials

Calcium alginate was prepared from sodium alginate, extracted from brown algae (medium grade, viscosity about 3,500 cP, i.e. 3.5 Pa s, for a 2% solution at 25°C, Sigma–Aldrich, Poole, Dorset, UK) and calcium chloride dihydrate (Sigma–Aldrich, Poole, Dorset, UK). The hydrogel was mixed with HA (Fluka Chemica, Buchs, Switzerland) to make the composite material (see below). These sources of alginate and HA are not approved for clinical use but were suitable for the experimental study described here.

2.2 Particle size

A Coulter counter (Model LS-230, Beckman Coulter, High Wycombe, Bucks, UK) was used to measure the particle sizes for the HA powder, as supplied and after the further processing described below. Samples of the powder were also examined by scanning electron microscopy (SEM). A thin layer of powder was mounted on a specimen stub and coated with gold for examination in the microscope (Model JSM-6060LV, JEOL, Tokyo, Japan) with an operating voltage of 10 kV.

HA powder was passed through a sieve (Endecotts, London, England) to produce particles whose diameters were less than 53 μm. Smaller particles were prepared using a ball mill (Szegvari Attritor System, Union Process, Akron, Ohio, USA). HA (75 g) was mixed with deionised water (75 cm^3) and milled (at 200 rpm) using ceramic balls (75 cm^3, diameter 5 mm). Two size ranges were obtained by milling for different times (10 min and 72 h). The slurry was drained, excess water removed (using a pipette) and the slurry heated (90°C for 24 h) to produce a concentrated suspension. Attempts to produce larger particles by sintering and sieving led to wide bimodal distributions of particle sizes when investigated using a Coulter counter and SEM.

2.3 Preparation of composite

Sodium alginate (1.5 g) was mixed with deionised water (25 cm^3), to produce a viscous solution, in a polypropylene beaker (capacity 100 cm^3). An ultrasonic cleaning bath (Ultrawave, Cardiff, UK) was used (for 5 min) to remove air bubbles. HA was suspended in deionised water (10 cm^3) and the ultrasonic bath was used to ensure that it was evenly dispersed. For the smaller HA particles, that were stored suspended in water, allowance was made for the mass of water in the original suspension when producing the suspension described here. The HA suspension was added to the sodium alginate solution and mixed (for 5 min). Calcium chloride dihydrate solution (1.2 g in 12 cm^3 deionised water) was added to the mixture. The beaker was covered in Clingfilm and left (for 48 h) to ensure that gel formation was complete. Materials were made with the following compositions (expressed as mass fractions of HA) 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8. Calcium alginate hydrogel (i.e. a composite with an HA mass fraction of zero) was also prepared by the procedure described above.

Samples for mechanical testing were cut from a cylinder of hydrogel (diameter 16.0 ± 0.5 mm) using a metal stamp. Slices (thickness 5.0 ± 0.5 mm) were cut from this cylinder using a scalpel and a device designed to ensure that parallel cuts were obtained. These test pieces had comparable dimensions to those that have been used previously [15].

2.4 Viscoelastic properties

A materials testing machine (ELF 3200, Bose Corporation, ElectroForce Systems Group, Minnetonke, MN, USA) equipped with a 225 N load cell (nominal precision ±0.005 N) was used for all the tests described in this paper. The machine was operated using WinTest Dynamic Mechanical Analysis software (Bose Corporation, ElectroForce Systems Group, Minnetonke, MN, USA). In all tests, specimens were immersed in a solution of calcium chloride dihydrate (at the same concentration used to prepare the calcium alginate gel) at room temperature (about 25°C) to prevent fluid loss.

Cyclic tests were performed, usually on three (but sometimes two or four) samples, for each of the eight composite compositions, each with three HA particle sizes (i.e. about 70 composite samples) and on calcium alginate hydrogel. In these tests a sinusoidal load, oscillating 1 N either side of a mean compressive load of 2 N, was applied to the specimen; seven loading cycles were applied at 23 frequencies, starting at 20 Hz and decreasing to 0.001 Hz. Rest periods (2 min) were allowed between each set of seven loading cycles, to enable the specimen to recover. Before data collection, specimens were subjected to eight sets of seven loading cycles (each followed by a rest period); the final set of these pre-conditioning cycles was applied at a frequency of 20 Hz and followed by a 5 min rest period. The WinTest software then calculated the storage, E', and loss, E'', moduli at the different frequencies.

2.5 Statistics

For each composition of composite, mean values of E' and E'' were calculated for each loading frequency, f

(see Sect. 3.1 for further details). One of the four data sets for a mass fraction of 0.7 was omitted because its values for E' and E'' were very different to the others; the remaining three data sets were consistent and were used for the calculations described below. It is not clear why the fourth set was different from the others. Mean values were plotted against the logarithm (base 10) of f, measured in Hz, in order to observe effects over a wide range of frequencies. Smooth curves could be fitted through the data points using third-order polynomials, so that the moduli could be represented by

$$E' = a'_0 + a'_1(\log f) + a'_2(\log f)^2 + a'_3(\log f)^3 \quad (3)$$

and

$$E'' = a''_0 + a''_1(\log f) + a''_2(\log f)^2 + a''_3(\log f)^3. \quad (4)$$

All statistical calculations were performed using a spreadsheet (Excel 2003, Microsoft, Reading, UK).

3 Results

3.1 Particle size

Table 1 shows the dimensions of the three ranges of sizes (numbered in order of increasing size) for the HA particles. Mean and median are the same for ranges 1 and 2 (produced by milling) because they had a Gaussian distribution of particle sizes. Range 3 (produced by sieving) had a less symmetrical size distribution. These results are consistent with qualitative observations made using SEM. Since there was no consistent and convincing evidence for particle size having any effect on the values of E' or E'', results from ranges 1 and 2 were pooled because they contained particles of a comparable size; range 3 was omitted because it did not have a symmetric size distribution.

3.2 Effect of frequency

Figure 1a shows a typical set of results, obtained for a composite with a mass fraction of HA of $m_f = 0.6$, showing how E' and E'' depend on frequency, f. The lines passing through the data points are third order polynomials. Above $\log f$ values of about -1.3, i.e. at a frequency of about 0.05 Hz, E' has a systematically higher value than E''. It can also be seen that E' increases with frequency. There is a slight increase in E'', at low f values, but it has a low, broad maximum at a $\log f$ value of about -1.5, i.e. at a frequency of about 0.03 Hz. This maximum is not apparent for all compositions; for others, E'' continues to increase slowly. Figure 1b shows the same data but presented as $|E^*|$ and tan δ, calculated from E' and E'' using Eqs. 1 and 2. $|E^*|$, which represents the overall stiffness of the composite, increases with f in the same way as E'. Tables 2 and 3 give the coefficients of the polynomials (Eqs. 3 and 4) that can be used to calculate E' and E'' for composites with each of the mass fractions investigated. $|E^*|$ and tan δ can then be calculated using Eqs. 1 and 2.

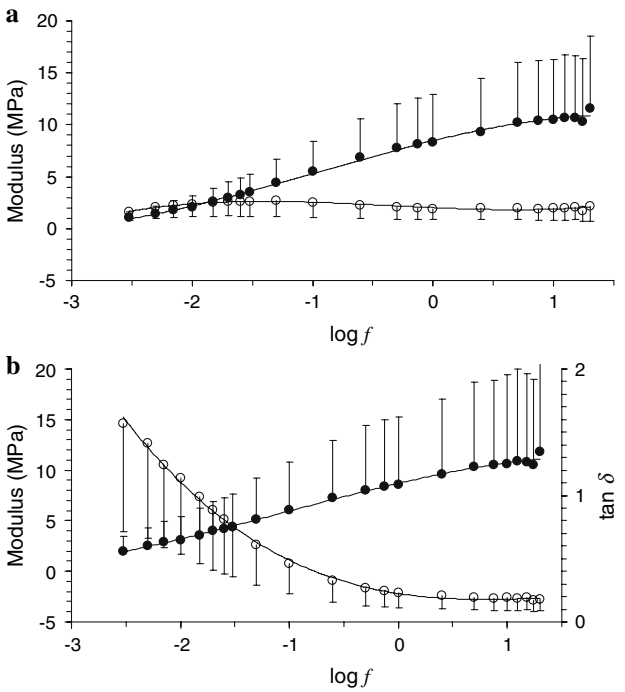

Fig. 1 Values of (**a**) storage, E', (filled circles) and loss E'' (open circles) moduli and (**b**) $|E^*|$ (filled circles)and tan δ (open circles) plotted against the logarithm (to the base 10) of loading frequency. Error bars represent standard deviations; upper bars only are shown for E' (and $|E^*|$) and lower bars only for E'' (and tan δ), to make the graphs clearer. Lines are the third order polynomials that gave the best fit to the data. This example is for a composite with an HA mass fraction of 0.6

Table 1 Particle sizes for HA as measured using a Coulter counter

Range number	Median size (μm)	Mean size (μm)	Modal size (μm)
1	2.3	2.3	2.5
2	4.0	4.0	4.1
3	10.1	8.6	11.3

3.3 Effect of composition

It is clear, from Tables 2 and 3, that increasing the proportion of HA in the composite increases its values for E' and E''. Figure 2 shows that a Reuss model [16] underestimates but provides approximate values for $|E^*|$. In this

Table 2 Coefficients of the polynomials fitted to E' plotted against $\log f$ for each composite, characterised by its mass fraction, m_f, of HA

m_f	V_f	a'_0	a'_1	a'_2	a'_3	R^2
0	0.000	3.4583	0.8403	−0.0637	−0.0307	0.997
0.1	0.036	4.8502	0.7427	−0.0059	0.1102	0.990
0.2	0.077	4.7455	0.7248	−0.0057	0.1138	0.994
0.3	0.125	5.6786	1.1039	−0.148	0.0633	0.995
0.4	0.182	7.0029	1.6906	−0.3107	−0.0079	0.999
0.5	0.250	8.1963	2.2958	−0.3403	−0.0285	0.998
0.6	0.333	8.4735	2.8289	−0.5217	−0.182	0.996
0.7	0.438	8.7763	3.5275	−0.202	−0.1469	0.995
0.8	0.571	9.8804	4.9071	0.2969	−0.1413	0.998

A volume fraction, V_f, of HA was also calculated, assuming a density of 1.0 g.cm^{-3} for calcium alginate, since it is mostly water, and 3.0 g cm^{-3} for HA [24]. These coefficients can be used to calculate values for E' from Eq. 3. R^2 is the square of the correlation coefficient that shows how well the polynomial curve fits the data; for a perfect correlation, it has a value of 1

Table 3 Coefficients of the polynomials fitted to E'' plotted against $\log f$ for each composite, characterised by its mass fraction, m_f, and volume fraction, V_f (see Table 2), of HA

m_f	V_f	a''_0	a'_1	a''_2	a''_3	R^2
0	0.000	0.6429	−0.0349	0.0737	−0.0178	0.976
0.1	0.036	0.9389	−0.271	0.227	0.0521	0.858
0.2	0.077	0.8886	−0.215	0.256	0.0482	0.878
0.3	0.125	1.181	−0.378	0.2688	0.1067	0.853
0.4	0.182	1.5798	−0.5183	0.2516	0.1425	0.907
0.5	0.250	1.8266	−0.526	0.3116	0.1959	0.868
0.6	0.333	2.0167	−0.5053	0.1964	−0.182	0.996
0.7	0.438	2.376	−0.2449	0.0931	0.1471	0.927
0.8	0.571	3.2557	0.498	0.1271	0.005	0.969

These coefficients can be used to calculate values for E'' from Eq. 4. R^2 is the square of the correlation coefficient (see Table 2)

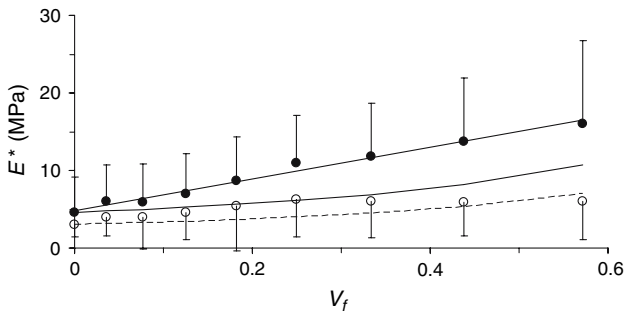

Fig. 2 Values of $|E^*|$ plotted against the volume fraction, V_f, of HA for loading frequencies of 0.1 Hz (open circles) and 20 Hz (filled circles); error bars represent standard deviations. The dashed curve represents the predictions of the Reuss model for the lower frequency; the continuous curve represents the predictions for the higher frequency. The straight line is the best linear fit through the filled circles

figure, $|E^*|$ is plotted against the volume fraction, V_f, of HA (see Table 2) rather than E' and E'', because $|E^*|$ is a measure of the overall stiffness of the composite. In all cases, the predictions of the Reuss model pass through the error bars associated with the data points. The level of agreement between the predictions of the Reuss model and experimental observations are better at the lower (0.1 Hz) of the two frequencies shown than at the higher (20 Hz) frequency. Similar levels of agreement were obtained at intermediate frequencies; at frequencies below about 0.1 Hz, the effect is slight.

4 Discussion

Our results show that, although HA can be used to stiffen calcium alginate hydrogels, the resulting stiffness is not much greater than that of the original gel because the composite behaves very similarly to a Reuss solid. The effect is greatest at higher loading frequencies (above about 5 Hz) when $|E^*|$ can increase by a factor of about 3.5 times when the mass fraction of HA in the hydrogel is 0.8. Figure 2 shows both the effect of increasing the mass fraction of HA and of increasing the frequency. The polynomial coefficients a'_0 (Table 2) and a''_0 (Table 3) increase with the mass fraction of HA in the composite, showing that both E' and E'' increase with increasing HA content (since $a'_0 > a'_1 > a'_2 > a'_3$ and $a''_0 > a''_1 > a''_2 > a''_3$ and, by definition the mass fraction of HA must be less than 1); a'_0 and a''_0 represent the values of E' and E'', respectively, when the frequency $f = 1$ Hz.

The effect of loading the calcium alginate gel with HA particles whose dimensions are of the order of a few micrometres can be calculated from empirical equations that are third-order polynomials. Tables 2 and 3 give the coefficients that enable E' and E'', respectively, to be calculated from the logarithm (to the base 10) of the loading frequency for a range of HA mass fractions. However, the values of these coefficients apply to alginates whose viscosity is comparable to the material used for our experiments. Figure 2 shows, for the example of a loading frequency of 20 Hz, that the data points can be fitted very closely by a straight line (in this case with an R^2 value, see Table 2, of 0.984). Therefore, E' and E'' for composites intermediate in composition between those tabulated, can be calculated by linear interpolation [17]. Values for $|E^*|$ and tan δ can be calculated from the corresponding values for E' and E'', using Eqs. 1 and 2, respectively.

Figure 1a shows that E', but not E'', increases with increasing frequency until about 10 Hz ($\log f = 1$) when the graph flattens. Figure 1b shows that $|E^*|$, which is related to E' and E'' by Eq. 1, increases in much the same way as E' (because the value of E' is greater than that of E''). This increase in E' and $|E^*|$ with increasing loading

frequency is analogous to the glass transition, in which a material undergoes a transition from a soft material to a glassy material with increasing temperature [18]. For biomaterials, that are intended to operate at a fixed temperature of 37°C, the frequency dependent transition is more relevant than the analogous temperature driven transition [18].

Simple Reuss and Voigt models assume that the stiffness of a composite, as measured by $|E^*|$, do not depend on particle size; no such dependence was observed in our samples. $|E^*|$ depends on E'' (Eq. 1) and $E''/2\pi f$ is a measure of viscosity, for a linearly viscoelastic material [19]. The viscous drag on a spherical particle is proportional to its radius (Stokes' law [20]) so that some dependence of E'' on particle size might be expected. Although there are many experimental studies that show that the radius of reinforcing particles affects the value of $|E^*|$ [10, 15, 21–23], it appears that any effect is very slight for calcium alginate hydrogels reinforced by HA particles whose dimensions are of the order of micrometres.

5 Conclusions

The main conclusions on the properties of calcium alginate hydrogels reinforced with HA particles, in the size range 2–10 μm, are listed below.

Storage and loss moduli, E' and E'', are independent of HA particle size.

E' depends on frequency, f, in the range 0.001–20 Hz.

E' has a systematically higher value than E'' when f is greater than about 0.5 Hz.

E' continues to increase, non-linearly, with increasing f, in the range 0.001–20 Hz.

$|E^*|$, the magnitude of the complex modulus (of which E' and E'' are real and imaginary parts, respectively) increases linearly with the volume fraction, V_f, in the range 0–0.6.

Approximate values of $|E^*|$ can be predicted by a Reuss model.

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