

In situ polymerization into porous ceramics: a novel route to tough biomimetic materials

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A hydroxyapatite-based biomimetic composite, which is henceforth referred to as a synthetic bony material with high toughness characteristics, was prepared. It was obtained from a hydroxyapatite (HAp) skeleton with a relative porosity fraction of ≈ 32 vol %, prepared by cold-isostatic-press compaction, followed by a sintering process, leading to a hydroxyapatite structure containing percolated submicrometer porosity channels. The percolated pores were infiltrated with a liquid mixture of ϵ -caprolactam monomer and an initiator, before homogeneous *in situ* polymerization to 6-nylon within the fully percolated pore structure was induced thermally. The final composite consisted of a dense interpenetrated hydroxyapatite/6-nylon network in a fraction $\approx 68/30$ vol %. The work of fracture value of the hybrid composite was found to be comparable with those found in two natural materials (bovine femur and nacre), which were also investigated under the same testing conditions.

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1. Introduction

In the recent years, several research groups [1–3] have demonstrated the feasibility of *in vitro* techniques for the synthesis of biomimetic material structures. However, the superior sophistication of the biological route has not been paired yet and these techniques, so far, have not proved to be applicable for practical uses. In this paper we suggest an alternative route, based on an *in situ* polymerization process carried out into an inorganic skeleton with micrometer-sized porosity. This method enables the synthesis of biomimetic hybrid inorganic–organic composites in inorganic-phase-rich fractions and, while aiming at a relatively complex structural design, it is based on a simple and easily reproducible process. The unique architectures and hierarchical structures of natural materials continuously stimulate and inspire the design of new synthetic composites. In recent years, translating these “lessons from nature” into new materials development has constituted a rapidly growing field of interest within the materials science community. The field, being referred to as biomimetics, deals with the preparation of hybrid inorganic–organic composites structured in such a way to mimic biologic materials. This approach implies that the sophisticated synthesis mechanisms developed during ages of evolution and natural selection are conducted on a much faster and cost-effective scale to be viable for practical applications. A common characteristic of biologic materials such as bone [4], nacre [5], sea urchin tooth

[6] and other highly tough materials in nature is the strong interaction between the inorganic and the organic phases. This characteristic allows the organic phase to act as a strong binder film forming energy dissipating bridges [7, 8] (or stretching ligaments) across the faces of a propagating crack, thus leading to a remarkable toughness. In biological materials, this crack-bridging mechanism is governed by structures hierarchically designed at a nanoscale level. Such complexity has led to the common perception that, in considering which methods can be used to mimic natural design, *in situ* synthesis techniques should be adopted. Precipitation *in situ* of calcium carbonate or hydroxyapatite (HAp) into a polymeric matrix, for example, has been proposed as a novel synthetic route to biomimetic composites [9, 10].

Despite significant advances in understanding biological mineralization and developing new fabrication processes, by far the composites to date obtained by these methods are still *in embryo* for actual structural applications. Alternatively, our philosophy has been to challenge a more efficient synthesis route based on the *in situ* polymerization of the organic binder into an inorganic porous structure, rather than mimicking the *in situ* precipitation of inorganic filler into an organic matrix. We start from (relatively) simpler inorganic porous scaffold subsequently filled with a (liquid) binding organic matrix, which is eventually polymerized *in situ*. The advantages of the proposed approach reside in the greater potential in controlling the inorganic

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scaffold structure (i.e., compared to the up-to-date *in situ* precipitation methods) and in the fact that a larger degree of freedom is given in the choice of the organic network.

2. Experimental procedure

The *in situ* polymerization process is schematically illustrated in Fig. 1.

2.1. Preparation of porous hydroxyapatite

A HAp skeleton with a relative porosity fraction ≈ 32 vol% was first prepared by cold-isostatic-press compaction of spherical HAp powder (Taihei Chemical, Co., Nara, Japan, average diameter = 18 μm), followed by a traditional sintering process in air (1 h at 950 $^{\circ}\text{C}$). Sintering performed without applying pressure led to a HAp structure containing percolated submicrometer porosity channels. Details describing the porosity morphology are given elsewhere [11].

2.2. Monomer infiltration and *in situ* polymerization

Particular care during following process must be taken for complete removal of air and/or atmospheric physisorbed species from the percolated network prior to monomer infiltration, thus achieving complete polymerization at the end of the process.

After evacuating the porous sample to $\approx 10^{-5}$ Torr for several hours at high temperature, the percolated pores

were infiltrated with a liquid mixture of commercially purchased ϵ -caprolactam monomer and 6-aminohexanoic acid as an initiator upon gradually reestablishing ambient pressure using Ar gas. Then, homogeneous *in situ* polymerization to 6-nylon within the fully percolated pore structure was induced thermally upon heating up to 100 $^{\circ}\text{C}$ for 14 h. The final composite consisted of an almost fully interpenetrated HAp/6-nylon network structure, which was proved by comparing the density of the specimen before and after infiltration/polymerization. This comparison indicated a volume fraction of 6-nylon of $\approx 30 \pm 2\%$ in the composite.

2.3. Elastic constant and fracture mechanics characterizations

The Young's modulus of both HAp/6-nylon composite and bone was measured by the flexural resonant frequency method. The bone specimen was tested with bending it along its longitudinal axis. Specimens for fracture mechanics tests were parallelepipeds $3 \times 4 \times 20$ mm ($B \times W \times L$) in dimension, with a straight-through notch (relative length, $a_0/W \approx 0.5$) placed at their center. The notch was introduced by a diamond cutter (blade thickness 0.2 mm) and sharpened to a radius $\approx 5 \mu\text{m}$ by using a razor blade coated with fine diamond paste [12]. Bars for fracture experiments were randomly cut from the polymer infiltrated composite body. The load-displacement curves were monitored during stable crack propagation. The span of the 3-point flexure jig was 16 mm, and the cross-head speed was 0.1 mm/min. Cracks stably propagated through the specimens by virtue of a specially designed bending-type crack-stabilizer [13]. In some cases, cracks were arrested and crack profiles observed *in situ* by a scanning electron microscope (SEM) operating at 5 kV. Prior to SEM observation, the specimens were lightly coated by evaporated carbon to avoid charging during observation. For comparison, samples of bovine femur and bivalve oyster nacre (*Crassostrea Nippona*) were also tested in the same bending geometry. Care was taken in avoiding drying of these natural biomaterials before testing, in order to preserve unaltered the stretching behavior of their protein structures.

3. Results and discussion

A highly porous percolated inorganic structure can be pressure-infiltrated with an organic liquid monomer filling the percolated porosity network of the pre-sintered inorganic scaffold. Then, *in situ* polymerization of the monomer is induced by addition of a suitable Initiator and by providing the necessary amount of heat required for the process. The resulting material is an inorganic-organic interpenetrated network, which resembles a biologic structure in a more simplified version. However, apart from this general precaution, the method easily allows the synthesis of materials with properties comparable or even improved over biological materials. As a term of comparison with natural materials, HAp-based biomimetic composite, which is referred to as a synthetic bony material with high toughness characteristics, was prepared.

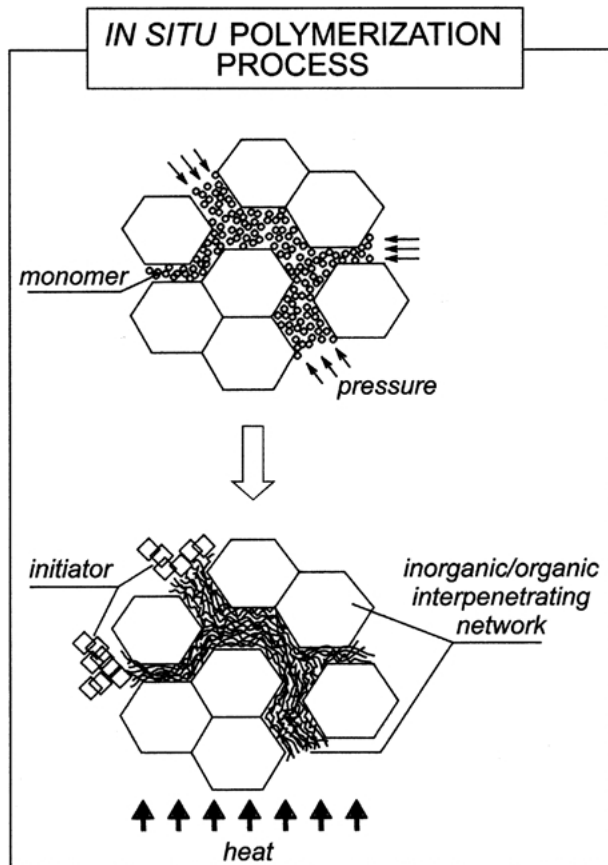


Figure 1 Schematic description of the *in situ* polymerization process into a porous ceramic skeleton.

For a preliminary structural evaluation of the composite, it is interesting to compare the Young's modulus of the present HAp/6-nylon with that of bone, which is a composite structure of HAp and collagen in a volume fraction slightly higher than that of 6-nylon in our synthetic composite. The Young's modulus of the HAp/6-nylon composite displayed a value of 55 ± 5 GPa, while that of bovine femur (along its longitudinal direction) was measured as 32 ± 6 GPa. Bonfield *et al.* [14] have reported a Young's modulus of ≈ 13 GPa for a composite consisting of HAp added with 40 vol % polyethylene. The present composite shows a relatively higher stiffness which, according to further optimizations of the *in situ* polymerization process, has to be adjusted to match exactly that of bone, if the composite has to be actually used for bone replacement. The Young's modulus of dense HAp has been reported to be ≈ 110 GPa [15]. If we assume a simple rule of mixture and a Young's modulus of 2.7 GPa for highly aligned 6-nylon [16], the calculated value of Young's modulus for the composite overestimates the experimentally obtained value by $\approx 40\%$. This may be due to either an incomplete interfacial bonding between HAp and 6-nylon or to a low alignment of the 6-nylon structure within the pores, or to both the above factors. The Raman microprobe experiments described afterward will provide further insight in the degree of alignment of polymeric nylon.

The results of the fracture mechanics characterization are shown in Fig. 2. A common characteristic of the load-deflection ($P-u$) curve of the three investigated materials is their stable (or semi-stable) fracture propagation, without a complete catastrophic fall of the $P-u$ curve upon displacement increase. A measure of the toughness of the material is the work required to stably break the bend specimen (referred to as work of fracture, W , henceforth), which is represented by the area left under the $P-u$ curve divided by twice the nominal cross-

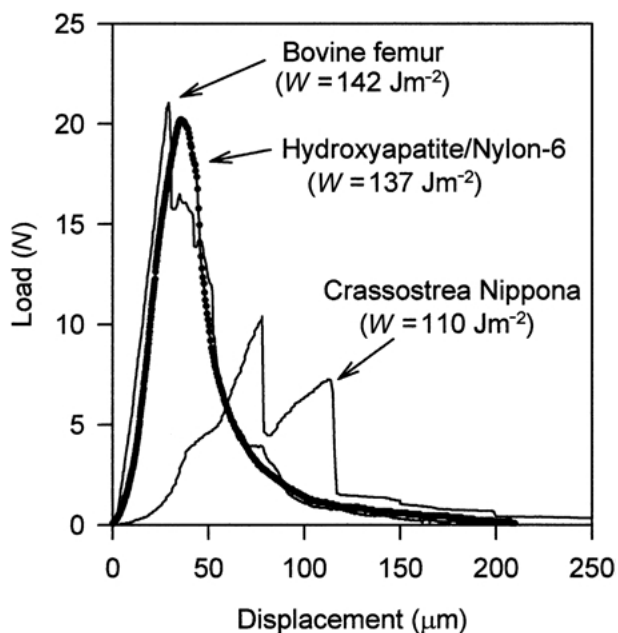


Figure 2 Load-displacement curve of the hydroxyapatite/6-nylon interpenetrating network composite is compared with that of bovine femur and bivalve oyster nacre (*Crassostrea Nippona*).

sectional area of the fractured surface [17]. The work of fracture value measured for the 68:32 vol % interpenetrated HAp/nylon-6 composite was $W = 137 \pm 20$ J/m², as an average value of five tested specimens. Such a W value was comparable with those found in the two natural materials also investigated under the same testing conditions [18, 19] ($W = 142 \pm 40$ and $W = 110 \pm 20$ J/m² for bovine femur and nacre, respectively, as also shown in Fig. 2). Note that the measured W values are about one order of magnitude larger than the intrinsic work of fracture found in highly covalent ceramics, according to the same testing procedure/geometry [20]. The important implication behind both the stable fracture behavior and the relatively high work of fracture is the presence of a microscopic toughening mechanism capable of significant extra-energy adsorption in the neighborhood of the propagating crack. To clarify the origin of such a mechanism in the newly developed HAp/6-nylon composite, the fracture phenomenon was observed *in situ* by loading a notched specimen in a jig placed into the SEM. The result of this observation is shown in Fig. 3A. A continuous, dense procession of polymeric ligaments (with a submicrometer diameter) pulled across the crack profile could be clearly envisaged. Results of a Raman microprobe characterization carried out *in situ* on the crack profile are also shown in the inset of Fig. 3A. Raman peaks of both HAp and 6-nylon are clearly recognized in comparison with Raman spectra collected on bulk materials [21, 22].

Stretching of microscopic polymer ligaments to form elastoplastic bridging sites should adsorb a remarkable amount of energy during fracture propagation, thus counteracting the brittle character of fracture propagation. This bridging mechanism, similar to that observed in natural materials [7, 8] (Fig. 3B and C), can fully explain the high work of fracture value measured, provided that a high alignment degree of the polymeric binder is achieved. It is known that the mechanical properties of polymeric nylon greatly depend on the internal degree of alignment of its macromolecules. A matter of concern in our investigations was to check whether polymerization within the narrow pores of the HAp network can actually produce some natural molecular alignment. To evaluate the inherent strength of the network, Raman microprobe monitoring of the nylon spectrum of bridging ligaments was performed *in situ* during stable fracture propagation. Under stress (cf. Fig. 4), a shift (relative to the zero-stress state) of selected Raman bands should be revealed, which is proportional to the local stress magnitude achieved within the probed microscopic volume. This microprobe spectroscopy technique has been recently applied to quantify the magnitude of microscopic bridging stresses developed in advanced ceramics [23]. *In situ* characterization of bridging stress by Raman microprobe spectroscopy in the present 6-nylon ligaments is conceptually similar to the direct strength measurement of the organic macromolecules of nacre, which was recently made using *in situ* atomic force microscopy (AFM) [24]. The Raman band of 6-nylon located at 1126 cm^{-1} (one of the two bands due to carbon skeletal stretching mode [21]) clearly showed a continuous shift upon increasing the externally applied load on the

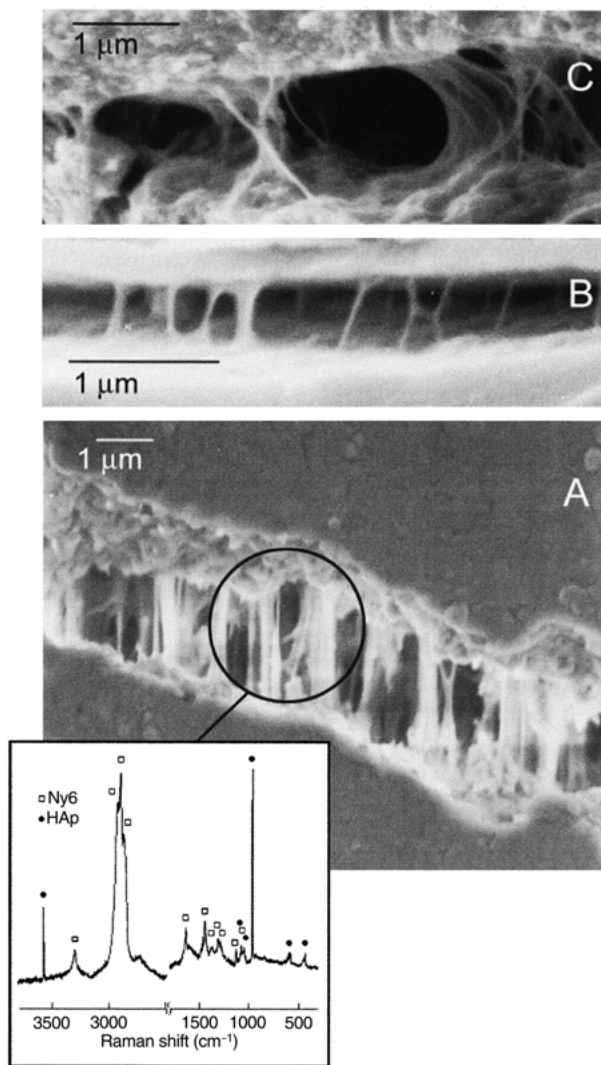


Figure 3 (A) Crack profile in the HAp nylon material observed *in situ* by SEM. Note the microscopic 6-nylon ligaments stretched across the opening crack faces. The inset shows a Raman microprobe spectrum detected within the microscopic encircled region, which reveals the presence of both HAp and polymerized 6-nylon phases; (B) protein ligaments stretched across the crack faces during fracture of *Crassostrea Nippona* naacre; (C) stretched collagen ligaments across the crack faces in bovine femur.

fracture mechanics specimen. Such a frequency shift, $\Delta\nu$, can be converted into stress according to a piezo-spectroscopic coefficient, $\Pi_u = -7.3 \text{ cm}^{-1}/\text{GPa}$, previously obtained by calibrating the Raman shift of bulk 6-nylon under known uniaxial stress/strain fields. A plot of the tensile stress, $\sigma = \Pi_u \Delta\nu$, measured *in situ* in the stretched 6-nylon ligaments of the biomimetic composite upon external loading, is shown in Fig. 4. The maximum shift, $\Delta\nu_{\text{max}} \approx -0.80 \text{ cm}^{-1}$, corresponds to a tensile strength, $\sigma_{\text{max}} \approx 110 \text{ MPa}$, on the bridging site, as shown in the inset. A comparison of the stress/strain curve of Fig. 4 with that reported for highly oriented bulk 6-nylon suggests that the 6-nylon network obtained by *in situ* polymerization (into porous HAp) owns a relatively high degree of molecular alignment and, thus, high strength. The Young's modulus of the 6-nylon polymerized *in situ* (i.e., the slope of the dotted line in Fig. 4) corresponds to a value $\approx 1.3 \text{ GPa}$ against the value 2.7 GPa reported in literature for highly aligned 6-nylon [16].

Smith *et al.* [24] measured a maximum tensile force

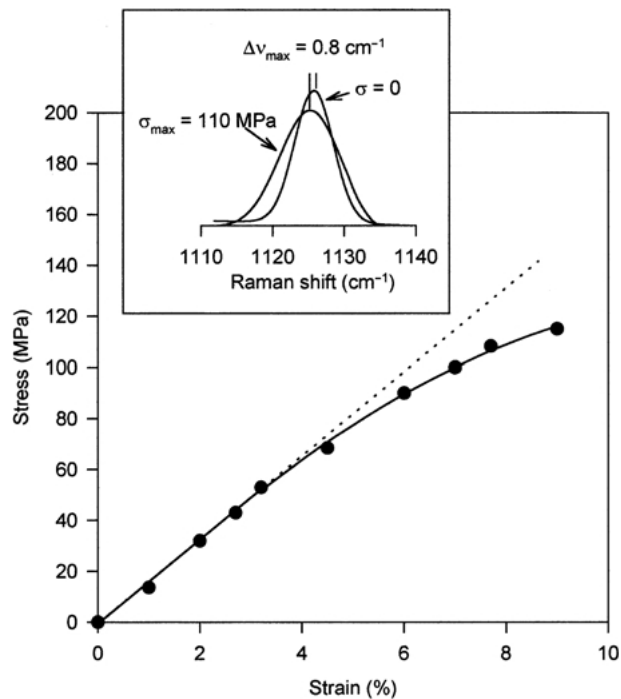


Figure 4 Stress/strain curve of 6-nylon ligaments as determined *in situ* upon external loading according to the shift of the 1126 cm^{-1} nylon peak. The inset shows the maximum peak shift which corresponds to a tensile strength of $\approx 110 \text{ MPa}$. The Young's modulus of the 6-nylon polymerized *in situ* (i.e. the slope of the dotted line) corresponds to a value $\approx 1.3 \text{ GPa}$ against the value 2.7 GPa reported in literature for highly aligned 6-nylon [16].

$\approx 400 \text{ pN}$ upon stretching *in situ* in the AFM a bridging molecule in naacre. Assuming an average ligament diameter $\approx 0.3 \mu\text{m}$ (i.e., as determined from scanning electron micrographs), the maximum tensile force involved in stretching a 6-nylon ligament across a propagating crack in the present biomimetic composite can be estimated to be $\approx 2 \times 10^4$ times larger than the AFM value of the naacre bridging protein. However, despite the four orders of magnitude higher tensile strength, the number of stretched 6-nylon ligaments per unit fracture surface area in the synthetic composite is significantly smaller that of the protein ligaments in naacre, due to the very fine lamellar structure of the natural material [5, 7, 8]. Thus, taking into consideration both fracture mechanics and structural stereology, the higher strength, but smaller number per unit volume, of the microscopic 6-nylon network as compared to the protein network in naacre, may justify the comparable toughness values measured.

In situ polymerization process to form organic networks, which interpenetrate porous inorganic structures, has potential for further improvement. For example, we experimentally succeeded in substituting collagen with polymeric nylon in bovine bone structures. We believe that this will open new perspectives in the fabrication of implant materials. On the other hand, when synthetic HAp is used, challenges for future design of tougher composites are both that of improving the porosity architecture of the inorganic skeleton and solving the *in situ* chemistry to produce polymeric networks with higher inherent strength (e.g. Kevlar). The present study clearly shows that, although the microscopic architecture of human manufactured composites

will still remain behind that of natural materials, mimicking natural microstructures while using strong synthetic molecules may lead to new engineering materials, with toughness at least comparable to the toughest materials available in nature.

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References

1. I. A. AKSAY and M. SARIKAYA, in "Ceramics Toward the 21st Century, Centennial International Symposium", edited by S. Soga and A. Kato (Ceramic Society of Japan, Tokyo, 1991) pp. 136–149.
2. P. CALVERT, in "Biomimetics: Design and Processing of Materials", edited by M. Sarikaya and I. A. Aksay (AIP Press, New York, 1995) pp. 145–161.
3. S. MANN, *Nature* **365** (1993) 499.
4. J. D. CURREY, *J. Biomech.* **23** (1990) 837.
5. M. SARIKAYA, J. LIU and I. A. AKSAY, in "Biomimetics: Design and Processing of Materials", edited by M. Sarikaya and I. A. Aksay (AIP Press, New York, 1995) pp. 35–90.
6. H. L. SOBEL and W. S. WILLIAMS, *J. Am. Ceram. Soc.* **80** (1997) 1706.
7. J. D. CURREY, *Proc. R. Soc. London B* **196** (1977) 443.
8. A. P. JACKSON, J. F. V. VINCENT and R. M. TURNER, *ibid.* **234** (1988) 415.
9. P. CALVERT and S. MANN, *J. Mater. Sci.* **23** (1988) 3801.

10. N. ALMQVIST, N. H. THOMSON, B. L. SMITH, G. D. STUCKY, D. E. MORSE and P. K. HANSMA, *Mater. Sci. Eng. C7* (1999) 37.
11. A. NAKAHIRA, M. TAMAI, D. MURAKAMI, S. MIKI and G. PEZZOTTI, *J. Austr. Ceram. Soc.* **36** (2000) 63.
12. T. NISHIDA, Y. HANAOKI and G. PEZZOTTI, *J. Am. Ceram. Soc.* **77** (1995) 606.
13. T. NISHIDA, Y. HANAOKI, T. NOJIMA and G. PEZZOTTI, *ibid.* **78** (1995) 3113.
14. W. BONFIELD, M. D. GRYNPAS, A. E. TULLY, J. BOWMAN and J. ABRAM, *Biomaterials* **2** (1981) 185.
15. J. L. KATZ, *J. Biomechanics* **4** (1971), 455.
16. J. ZIMMERMANN, in "Concise Encyclopedia of Polymer Science and Engineering", edited by J. I. Kroschwitz (John Wiley & Sons, New York, 1990) pp. 748–753.
17. H. G. TATTERSALL and G. TAPPIN, *J. Mater. Sci.* **1** (1966) 296.
18. The work of fracture of toughened materials is a parameter strongly dependent on the area of the fractured specimen surface, the larger the fractured surface area the larger the measured W value. The W values measured in the present fracture mechanics geometry should be considered to be comparatively meaningful referring to small fractured areas ($< 2 \text{ mm}^2$).
19. J. D. CURREY, P. ZIOUPOS and A. SEDMAN, in "Biomimetics: Design and Processing of Materials", edited by M. Sarikaya and I. A. Aksay (AIP Press, New York, 1995) pp. 117–144.
20. G. PEZZOTTI, I. TANAKA and T. NISHIDA, *Phil. Mag. Lett.* **67** (1993) 95.
21. P. J. HENDRA, D. S. WATSON, M. E. A. CUDBY, H. A. WILLIS and P. HOLLIDAY, *Chem. Comm.* (1970) 1048.
22. V. SERGO, O. SBAIZERO and D. R. CLARKE, *Biomaterials* **18** (1997) 477.
23. G. PEZZOTTI, *J. Raman Spectrosc.* **30** (1999) 867.
24. B. L. SMITH, T. E. SCHAFFER, M. VIANI, J. B. THOMPSON, N. A. FREDERICK, J. KINDT, A. BELCHER, G. D. STUCKY, D. E. MORSE and P. K. HANSMA, *Nature* **399** (1999) 761.

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