

Chapter 2

Case Study: Hydroxyapatite–Titanium Bulk Composites for Bone Tissue Engineering Applications

2.1 Background: Brittleness of Hydroxyapatite

Healthy bone and joints (e.g. articulating, knee, and hip joints) are necessary for the structural stability and pain free movements. However, these healthy bones and joints can get damaged in some unfortunate cases due to accident/diseases and fail to perform their normal functions. The damaged bones can be replaced or repaired with allogeneic bones or allografts [1–5]. However, the associated risk of transmission of life threatening infections with allogeneic bones is significant particularly due to inferior osteoconductivity as well as poor mechanical properties of the freeze dried [1–3, 6–13]. In contrast, the designed biomaterials with bone-mimicking properties can be used as an alternative to the allografts and autografts.

Biomaterials development has a long history. In 1940, Jean and Robert Judet of Paris performed the replacement arthroplasty to replace the femoral head with acrylic resin, which opens up the possibility of joint replacement [14]. However, after an initial success, this implant failed due to its poor mechanical properties as well as inapt design. More than 10 decades later, when most of the issues related to joint replacement have been settled down, the interest in new biomaterials remain as fierce ever. A biomaterial is a synthetic biocompatible material/device that has been intently designed to persuade a definite activity in the biological system, which can simulate the desired biological function, without generating a short and/or long term damage to the nearby or distant tissue as well as without being getting damaged in this procedure [15–17].

For many biomedical applications, a biomaterial should show the bioactivity, osteoconductivity, and osteoinductivity. Bioactivity is defined as the property of implant material to make direct bonding with living bone [18–21]. The bonding is mediated by an intervening (apatite) layer formation between the implant and the bone [22]. Osteoconductivity is defined as the ability of implant material that can serve as a scaffold for new bone formation and therefore, promotes the osteoblast adhesion, proliferation, and differentiation [23, 24]. Osteoinductivity is the ability

of a biomaterial to induce the new bone formation, implanted at non bone forming site, *in vivo* [25].

It is now well recognized that in case of bone applications the success of an implant material depends on biocompatibility, osseointegration property, physical/mechanical properties as well as antibacterial property etc. [26–29]. Metals have an advantage of good mechanical properties like high fracture toughness and flexural strength. But the effects such as corrosion as well as wear (results in leaching of metal ions), and stress shielding results in irritation, inflammation, and loosening of implants [30, 31]. Additionally, bioinert nature of metal implants result in weak bonding with host bone, *in vivo* [32, 33]. Although ceramics based biomaterials are found suitable for hard tissue applications due to its biocompatibility, corrosion resistance, and compressive strength properties [34, 35], inherent brittleness of ceramics may result in the early stage failure of implants during load bearing applications [36]. A classic example of a brittle bioceramic is hydroxyapatite, which widely researched due to its structural similarity with major inorganic component of bone composition [36]. In the context of load bearing implants, high elastic modulus of the implant with respect to neighbouring bone causes stress shielding, which is responsible for bone resorption near to the implant [37–41]. Therefore, the elastic modulus of an implant ideally needs to be matched with bone, while efforts are to be made to enhance both fracture toughness and strength.

In the above backdrops, the hydroxyapatite (HA)-based metal or ceramic reinforced composites can be used as a potential bulk implant material, where second phase contributes specifically towards better mechanical properties. The choice of second phase is limited as it needs to have good mechanical property as well as biocompatibility. As compared to Al_2O_3 , ZrO_2 and stainless steels, titanium (Ti) is found to be more promising second phase material for orthopedic applications due to its excellent corrosion resistance property under physiological environment, low density (4.540 g/cc) as well as lower elastic modulus (~ 110 GPa) than Co–Cr alloy (~ 230 GPa)/stainless steel (~ 205 GPa) [26, 41] and some data are shown in Table 2.1. The design of HA–Ti composites underlies the fact that the Ti addition significantly improves the mechanical properties of the composites without degrading the cytocompatibility properties [49–51].

Table 2.1 A comparison of physical and mechanical properties of bone with some metallic and ceramic biomaterials [26, 42–48]

Properties	Cortical bone	Cancellous bone	HA	Ti	Al_2O_3	ZrO_2
Young modulus (GPa)	14–20	0.05–0.5	80–110	117	390	205
Compressive strength (MPa)	170–193	7–10	400–900	250–600	3900	3000
Fracture toughness ($\text{MPa m}^{1/2}$)	2–12	0.1	0.7–1.2	60	5.2	10
Apparent density (g/cm^3)	1.8–2.0	0.1–1.0	3.16	4.54	3.9	6

HA–Ti composites can be prepared by different sintering routes, such as pressureless sintering, pulse electric current sintering (PECS), spark plasma sintering (SPS) etc. It is difficult to retain the Ti in the composite by conventional pressureless sintering techniques. For example, Nath et al. conducted a number of pressureless sintering experiments on the HA–Ti system and reported the extensive formation of TCP and CaTiO_3 in the composites [52]. On the other hand the SPS as well as PECS techniques with suitably chosen sintering parameters will allow the sintering of HA–Ti composites with retention of almost all Ti in the sintered compacts [52–55]. Nakahira et al. developed the HA–Ti composites with Ti content of 20 and 25 wt%, using PECS technique and compared the results with hot pressed HA–Ti composites of similar compositions [53]. Extensive study by our group indicates that it is possible to retain the Ti in the composite after sintering. However, in limited studies on HA–Ti compositions [52–55], no comprehensive attempt has been made to understand the influence of Ti content on the mechanical and biocompatibility property, when both HA and Ti are retained in the sintered composites.

In developing a bone replacement material, the choice of the second phase depends on the application as and the desired functionality. For example, BaTiO_3 , CaTiO_3 , Fe_3O_4 , and ZnO or Ag can be added to HA as a second phase to introduce the piezoelectric, electrical conductivity, magnetic, and bactericidal properties, respectively [56–60]. However, brittle nature and the load bearing incapability of the ceramics may limit wider application of such types of ceramic–ceramic composites and some examples includes HA–20HA_{whisker}, HA–20ZrO₂, and HA–(Al_2O_3 coated) ZrO₂ with fracture toughness of 1.4, 1.5, and 3 MPa m^{1/2}, respectively [61–63]. Interestingly, a glaring example of HA-based ceramic composite with fracture toughness higher or equal to that of the cortical bone is still unavailable (see Fig. 2.1). In contrast, the incorporation of metallic reinforcement as second phase may improve the fracture toughness of the HA-based composites. However, the sintering reactions between HA and metal phase are of major concern and can affect the biocompatibility properties *in vitro* and *in vivo*. Although stainless steel and Co–Cr alloys are competitively better than ceramics counterpart in terms of fracture toughness, the high elastic modulus of metals however may lead to stress shielding during *in vivo* performance [29]. In contrast, Ti was found to be most suitable for biomedical applications due to excellent biocompatibility, corrosion resistance property and stability in physiological conditions [64]. Thus, in order to incorporate both load bearing capability without fracture under peak loading as well as osseointegration property, the HA–Ti composites can be used as a bulk biomaterials. Over the various composites (HA– BaTiO_3 , HA– CaTiO_3 , HA– Al_2O_3 , HA–ZrO₂, HA–ZnO, HA–Ag), the selection of HA–Ti composites as a model system in the present chapter can be justified on the basis of following aspects: (a) low density of HA (3.16 g/cc) [65] and Ti (4.54 g/cc); (b) high fracture toughness of metallic Ti (60 MPa m^{1/2}) [26]; (c) bioactivity of HA and bio-inertness of Ti; (d) corrosion resistant properties of Ti; (e) limited dissolution of HA under physiological condition [66].

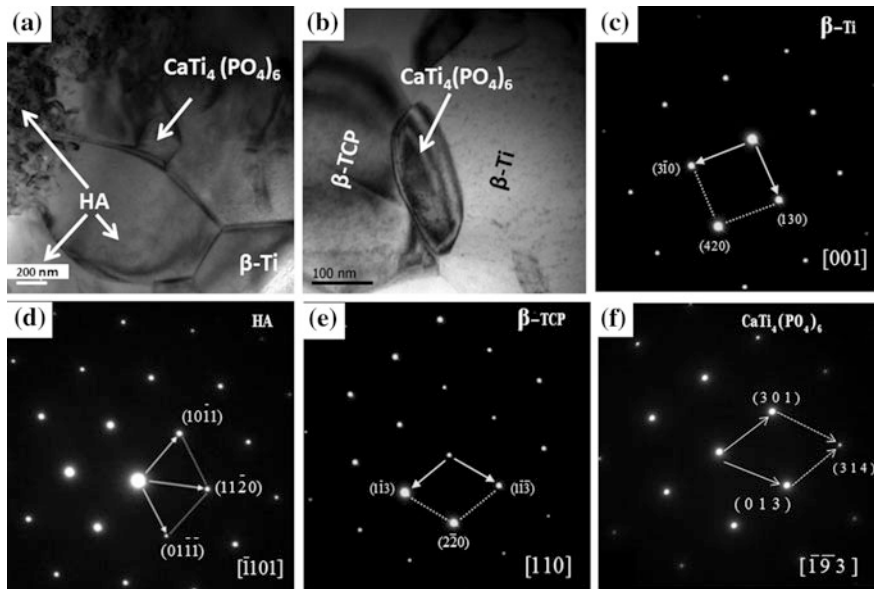


Fig. 2.1 Representative bright field TEM micrographs (a, b) of HA–20Ti composite, showing the different phases, formed during sintering. SAD patterns confirm the presence of β -Ti (c), HA (d), β -TCP (e), and $\text{CaTi}_4(\text{PO}_4)_6$ grains (f) [67]

2.2 Processing of HA–Ti Composites

HA–Ti composites can be prepared either by conventional pressureless sintering or advanced sintering, such as spark plasma sintering (SPS). SPS has the advantage of higher heating rate and shorter soaking time over conventional sintering. The advanced sintering techniques have many other advantages as compared to the conventional sintering.

Traditionally, HA–Ti composites were prepared by the homogeneous mixing of the HA and Ti powders using a ball-mill, followed by green compaction and then pressureless sintering at relatively higher temperature ($\geq 1100^\circ\text{C}$) and soaking time (≥ 1 h) [52]. Recently, the FAST (field assisted sintering technique) has been utilized to prepare dense compact from powder mixture within a short time [53, 67, 68].

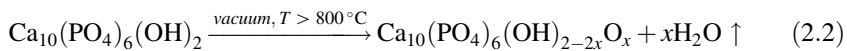
2.2.1 Conventional Sintering

In this subsection, the literature reports on conventional sintering of HA–Ti are briefly discussed. Fahami et al. [69] used CaHPO_4 , CaO , and Ti powders as precursors to prepare the HA–Ti composites by the mechanochemical synthesis of mixture of HA and Ti according to the following reaction:



It is important to note that the lower crystallinity of the HA powder results in the increased bioresorption and solubility under physiological conditions [70]. Therefore, further attempt has been made by Fahami et al. [69] to increase the crystallinity from 13 to 69 % by annealing the ball-milled powder at 650 °C for 2 h, synthesized after reaction (2.1). However, this results in the oxidation of Ti and decomposition of HA to β -TCP (beta-tri-calcium phosphate) phase. In a different approach, Chu et al. [71] prepared the HA–20 wt% Ti composite by mixing HA and Ti powders in a ball mill, followed by hot pressing at 900 and 1000 °C. Importantly, the sintering at 1000 °C leads to the formation of α -TCP and $\text{Ca}_4\text{O}(\text{PO}_4)_2$ phases. The difference in the linear thermal expansion coefficient (α) of HA ($17.3 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$) [72] and Ti ($8.4 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$) [73] phases results in inferior sinterability in case of HA–20 wt% Ti composite with ~ 67 % relative density, achieved when sintered at 1200 °C [71]. However, the same sintering conditions result in ~ 88 % relative density in monolithic HA. Importantly, the addition of Ti to HA leads to an increase in fracture toughness from 0.7 to 1.0 MPa m^{1/2}. Although Ti addition to HA was intended to increase the fracture toughness significantly, the marginal improvement in the fracture toughness can be attributed to poor sinter density of the composite. In an earlier work, Nath et al. [52] prepared the HA–Ti composites with varying amount of Ti (10–40 wt%) using pressureless sintering. It has been reported that the sintering of different compositions at 1000–1400 °C for 2 h leads to the extensive sintering reactions and therefore the formation of TiO_2 , CaO, TCP and CaTiO_3 (Table 2.2). The thermodynamic calculations show the higher probability of oxidation of Ti during conventional sintering due to higher sintering temperature and longer holding time [52]. Such calculations also indicate the possibility of reaction between HA and TiO_2 above 750 °C.

Considering the thermodynamical aspect, Yang et al. [55] prepared the HA–10 wt% Ti and HA–20 wt% Ti composites by sintering at 1100 °C in vacuum to minimize the oxidation of Ti. The FT-IR and XRD analysis of the HA–Ti samples show the decomposition of HA to α -TCP, TTCP (tetracalcium phosphate) as well as $\text{Ca}_2\text{Ti}_2\text{O}_5$. As the sintering temperature further increases, HA is reported to decompose to HA with deficiency of hydroxyl group in following way [74, 75]:



The water vapor produced from reaction (2.2) reacts with Ti to form TiO_2 [see Eq. (2.3)], which can finally react with HA to form β -TCP and CaTi_2O_5 [see Eq. (2.3)].

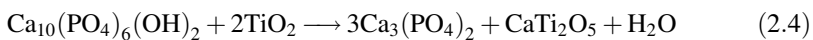
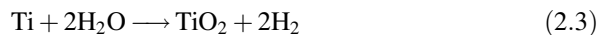


Table 2.2 XRD results, showing the effect of sintering parameters (pressure, sintering temperature, and soaking time) on the sintering reaction of HA–Ti composites [52, 53, 67]

	Conventional sintering (soaking time ≥1 h)				Advanced sintering (soaking time ≥5 min)		
	Pressureless sintering in air at temperature				Pulse electric current sintering in argon		Spark plasma sintering in argon
	1000 °C	1200 °C	1300 °C	1400 °C	900 °C	1000 °C	950 °C
HA	HA	HA	HA	HA, β-TCP, α-TCP, CaO	–	–	HA
HA–10Ti	HA, β-TCP, α-TCP, CaO, TiO ₂ , CaTiO ₃	HA, β-TCP, CaO, TiO ₂ , CaTiO ₃	HA, β-TCP, α-TCP, CaO, TiO ₂ , CaTiO ₃	HA, β-TCP, α-TCP, CaO, TiO ₂ , CaTiO ₃	–	–	HA, Ti, TiO ₂ , Ti ₂ O, β-TCP, CaO, CaTi ₄ (PO ₄) ₆
HA–20Ti	HA, CaO, TiO ₂ , CaTiO ₃	HA, β-TCP, CaO, TiO ₂ , CaTiO ₃	HA, β-TCP, CaO, TiO ₂ , CaTiO ₃	β-TCP, CaO, TiO ₂ , CaTiO ₃	HA, Ti ₃ O	HA, Ti ₃ O, Ti ₂ O	HA, Ti, TiO ₂ , Ti ₂ O, β-TCP, CaO, CaTi ₄ (PO ₄) ₆

Therefore, it is not impossible but extremely difficult to retain Ti in the sintered product using conventional sintering routes. The phase stability aspect in summarized in Table 2.2.

The sintering under a protective atmosphere (e.g. argon) is another promising approach towards retaining the metallic Ti in the sintered product. In order to see the effect of protective environment on the sintering reaction in the HA–Ti composites, Ning et al. [76] conventionally sintered the HA–50Ti composite at 1200 °C for 30 min in argon atmosphere. However, the sintering leads to the formation of Ti₂O, CaTiO₃, CaO and TiP-like phases along with α-Ti.

In summary, conventional sintering of HA–Ti composites even at very low temperature of 1000 °C leads to the extensive dehydroxylation of HA as well as reaction between HA and Ti. The formation of TCP phases will result in higher dissolution of the implant materials and therefore, decreased mechanical strength during service period, *in vivo*. As mentioned earlier, it is extremely difficult to retain the metallic Ti in the composite during the conventional sintering. The sintering in protective atmosphere of Ar was not found to be helpful in retarding the oxidation reactions in conventional sintering due to the longer sintering duration (≥1 h) and

higher holding temperature (≥ 1000 °C). In the following, we shall discuss the applicability of advanced sintering techniques (e.g. SPS) to prepare the composites with predominant retention of metallic Ti.

2.2.2 Advanced Sintering

Among various advanced sintering techniques, field activated sintering (FAST) is widely investigated to resolve the issues related to the conventional sintering, discussed earlier. For example, Nakahira et al. [53] reported the pulse electric current sintering (PECS) of HA–20 vol.% Ti and HA–25 vol.% Ti composites at 800 and 1000 °C under 30 MPa in a protective argon atmosphere. Although PECS suppresses the oxidation of Ti at 900 °C, the formation of Ti_2O_3 was found in samples sintered at 1000 °C. Mondal et al. [77] used the spark plasma sintering technique to sinter β -TCP and Ti powders at 1200 °C temperature under 50 MPa pressure. Despite the retention of β -TCP and Ti, this results in the formation of CaTiO_3 .

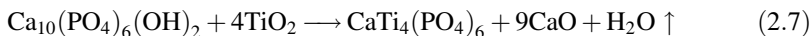
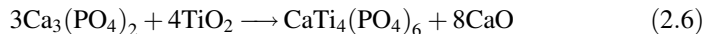
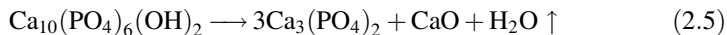
In a recent work, the author's research group has reported the results of extensive study on spark plasma sintering and phase evolution of HA–xTi ($x = 5, 10, 20$ wt%) composites [67]. The processing conditions were intelligently tailored to achieve high toughness with minimum sintering reaction between the HA and Ti. The XRD analysis confirms the predominant presence of HA and Ti.

In order to observe the finer scale microstructure of the sintered HA and HA–Ti samples as well as to identify the reaction product at HA/Ti interface, transmission electron microscope (TEM, FEI, UTWIN T-20) with an accelerating voltage of 200 kV was used. Figure 2.1a shows the representative bright field TEM micrograph of HA–20Ti composite, revealing the presence of different phases. The presence of these phases has also been confirmed at different locations in the same sample. Figure 2.1b represents the bright field micrograph of such a location. The detailed analysis of the selected area diffraction pattern (Fig. 2.1c–f) indicate the presence of β -Ti, HA, β -TCP, as well as the formation of $\text{CaTi}_4(\text{PO}_4)_6$ phase at the interface between HA and Ti. It is to be noted that β -TCP is a phase formed by the dehydroxylation of HA.

Overall, the critical analysis of SAD patterns reveals the formation of $\text{CaTi}_4(\text{PO}_4)_6$ as grain boundary phase, which is likely to be formed either due to limited sintering reaction between β -TCP and TiO_2 or between HA and TiO_2 . The presence of β -Ti also indicates the suppression of $\beta \rightarrow \alpha$ transformation of Ti phase during cooling through 882 °C (transformation temperature), perhaps due to faster cooling.

We shall now discuss the formation and morphology of finer scale sintering reaction product. It has been reported that dehydroxylation of HA does not occur at a sintering temperature below 1300 °C [52, 62]. In the presence of Ti and

Ti-oxides, HA starts to dissociate at a much lower temperature [55]. The detailed microstructural investigations of HA–Ti composites using TEM indicates the possibility of formation of reaction product between HA and Ti during sintering. Based on extensive TEM-SADP analysis, it has conclusively been shown that the reaction product to be $\text{CaTi}_4(\text{PO}_4)_6$, which is deemed to be the result of following reactions:



The reaction (2.10) is known as dehydroxylation of HA and leads to the formation of β -TCP. The reaction product $\text{CaTi}_4(\text{PO}_4)_6$ can form due to reaction (2.6) and/or (2.7). In view of the lack of data on Gibbs free energy of formation of related phases, any analysis on the thermodynamic feasibility of reaction (2.10–2.12) could not be made. Although TEM investigation does not reveal the presence of CaO phase, detailed analysis of XRD data shows the peaks corresponding to CaO to a detectable extent. Also, Ti_2O or TiO_2 could not be detected during TEM analysis of any of the HA–xTi samples.

2.3 Mechanical Properties

In the following, the mechanical properties of HA and HA–Ti composites are analysed. The hardness values shows a decreasing trend with addition of Ti to HA ($\text{HA} \approx 7.1 \pm 0.5$ GPa, $\text{HA-10Ti} \approx 6.1 \pm 0.6$ GPa). One can measure the plastic work of indentation by measuring the area under P–h curve (= area under loading curve – area under unloading curve) [78]. Such measurements indicate that the plastic work of indentation is higher than elastic work of indentation for both HA and HA–10Ti.

The elastic moduli, measured using nanoindentation are 94 ± 1.8 GPa for HA and 80.6 ± 0.9 GPa for HA–10Ti composite. The elastic modulus has also been measured using impulse excitation method. The measured values show a similar trend of the decrease of elastic modulus with Ti addition. The measured elastic moduli for different materials are as follows, $\text{HA} = 61.4 \pm 1.4$ GPa, $\text{HA-5Ti} = 58.1 \pm 0.2$ GPa, $\text{HA-10Ti} = 54.4 \pm 0.3$ GPa and $\text{HA-20Ti} = 50.2 \pm 0.8$ GPa [79]. Since the resonance

frequency method measures the elastic stiffness properties of a bulk sample, such results are more realistic and representative of the true modulus, rather than the nanoindentation results. The latter is based on the estimation from the slope of unloading response from an indented region of length scale of less than 1 μm .

In further characterization of mechanical properties, the flexural strength of the sintered composites has been measured using 3-point flexural test with 12 mm gauge length. The flexural strength systematically increases with increase in Ti-content in the composite. The flexural strength of pure HA is 70.7 ± 2.3 MPa. However, the maximum measured value of the flexural strength is measured as 99.7 ± 1.2 MPa in case of HA–20Ti composite. Therefore, the addition of Ti up to 20 wt% leads to about 41 % increase in the flexural strength. This shows a marked improvement in the flexural strength in HA–Ti biocomposite. It is to be noted here that the flexural strength of cortical bone is 150–170 MPa [80].

The fracture toughness of the sintered products have been measured using SEVNB test in 4-point flexural mode and a test sample is shown in Fig. 2.2b. As mentioned earlier, this technique is proved to be more acceptable than the indentation fracture toughness measurement technique in terms of providing reliable estimate of long crack fracture toughness. The SEVNB toughness results are plotted in Fig. 2.8a against amount of Ti addition to HA. For pure HA, the measured K_{IC} value is found to be 3.4 ± 0.2 MPa $\text{m}^{1/2}$. As compared to the monolithic HA, HA–10Ti exhibits about 35 % increase in the K_{IC} value (4.7 ± 0.1 MPa $\text{m}^{1/2}$). However, the addition of 5 wt% Ti does not show any significant improvement. On the other hand, 20 wt% Ti addition leads to about 26 % increase in K_{IC} value as compared to that of monolithic HA. K_{IC} value decreases in case of HA–20Ti sample as compared to HA–10Ti. Therefore, 10 wt% Ti has been found to be optimum Ti addition to HA to achieve substantial improvement in fracture toughness (see Fig. 2.2a). It is to be noted that the fracture toughness of the cortical bone is reported be in the range of 2–12 MPa $\text{m}^{1/2}$ ⁵⁸. Therefore, the present measurements are higher than the lower limit of fracture toughness of the cortical bone.

In order to investigate the statistical significance in terms of the property difference among the samples were used and results are summarized in Figs. 2.2, 2.3, and 2.4. Figure 2.8 shows the effect of Ti addition to HA on enhancement in fracture toughness (K_{IC}) and work of fracture (G_{IC}). Dunnett's t test shows the significant difference, when HA was compared with HA–10Ti and HA–20Ti samples for both K_{IC} and G_{IC} . However, no significant difference between HA and HA–5Ti samples was noted. Dunnett's C test found the significant difference between HA and HA–10Ti as well as between HA–5Ti and HA–10Ti in terms of both K_{IC} and G_{IC} .

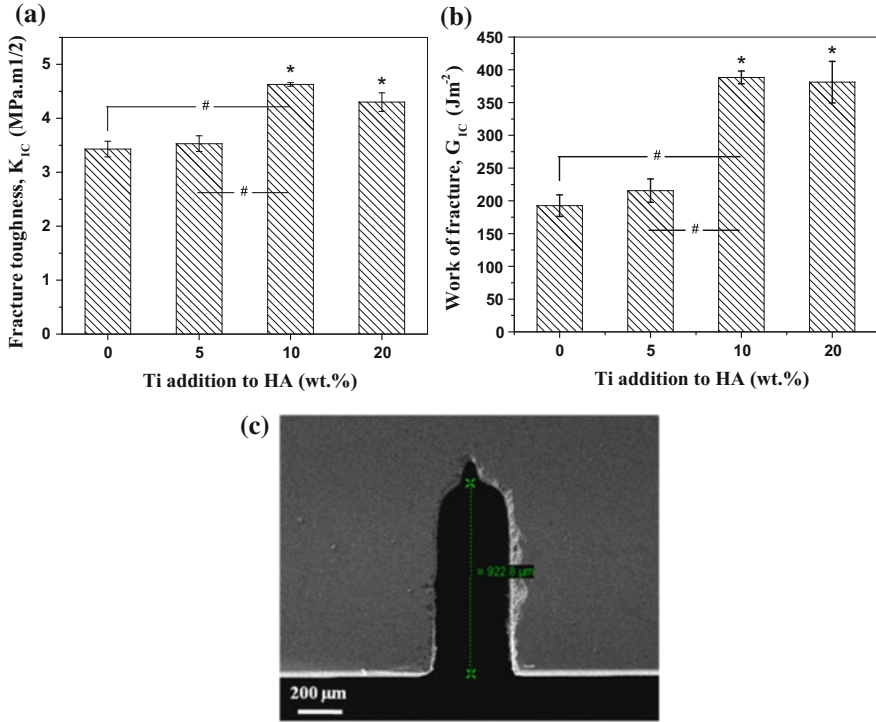
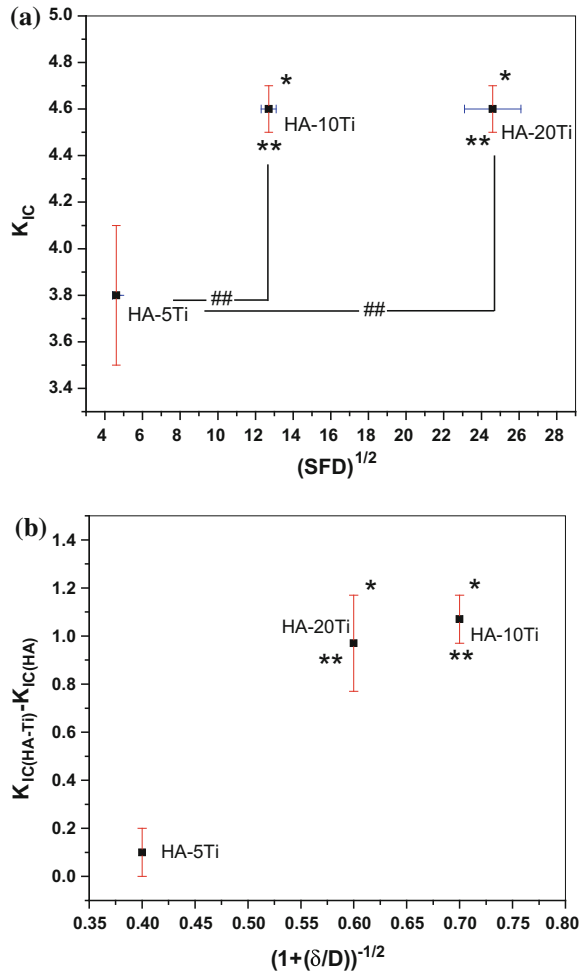


Fig. 2.2 Variation in fracture toughness (a) and work of fracture with wt% Ti in HA (b). Typical profile of V notch in one of the SEVNB test sample, as seen using SEM (c). Data in a and b has been represented as mean $\pm$ standard error. Dunnett's t (2 side) were used to compare the HA with HA–Ti samples (marked with *); while Dunnett's C test used to know the significant difference among all samples (marked with #). Statistical analysis shows the significant difference at 0.05 level for n = 5 [67]

Summarizing, the mechanical properties (hardness, flexural strength and fracture toughness) of spark plasma sintered HA–Ti composites show substantial improvement as compared to the properties obtained with earlier developed HA-based composites (see Fig. 2.1). Figure 2.4 compares the fracture toughness results of present investigation with other HA-based composites. We can analyse the results in terms of four categories: I (HA-0x), II (HA-5x), III (HA-10x), and IV (HA-20x), where x is the amount of the second phase. In all categories, Dunnett's t test (2 side) shows the significant difference, when K_{IC} from the present work is compared with earlier reported results. Also, various symbols, like *, **, #, ## indicate the

Fig. 2.3 Plot showing the variation in fracture toughness of HA–Ti composites versus fraction of particles nearby to the crack tip ‘S’, Ti particle size ‘D’ and volume fraction ‘F’. **a** Plot showing dependence of the toughness increment on Ti particle size ‘D’ and nearest neighbor distance for Ti, ‘ δ ’. In plot **a**, * and ** symbols show the significant difference between HA–5Ti and other HA–Ti samples for K_{IC} and $(SFD)^{1/2}$, respectively, when Dunnett’s t test was applied. The symbol, ## is showing the significant difference among the samples, measured by Dunnett’s C test. In plot **b**, * and ** showing the significant difference between HA–5Ti and other HA–Ti samples for $K_{IC(HA-Ti)} - K_{IC(HA)}$ and $(1 + (\delta/D))^{-1/2}$, respectively, when Dunnett’s t test was applied [67]



significant difference at 0.05 level. In the following section, the reason for such an increase by correlating the microstructure of the composite processed by novel SPS technique will be discussed with an effort to establish microstructure-property correlation.

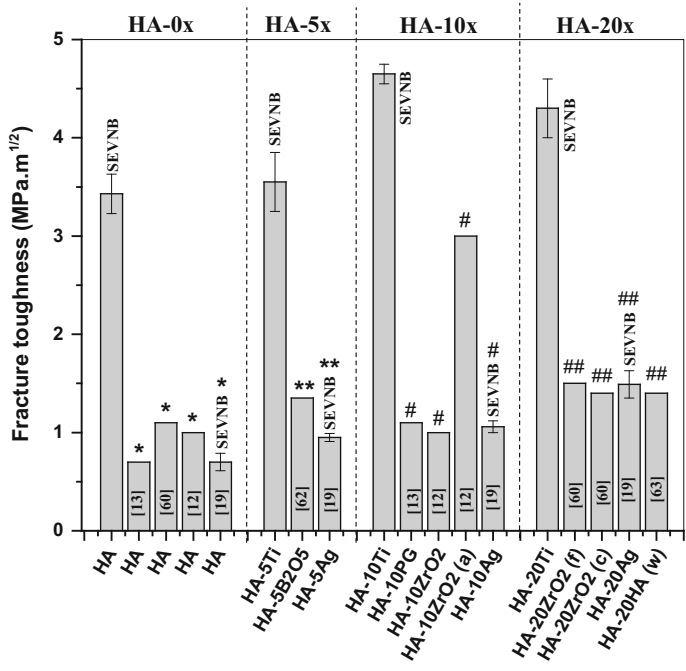


Fig. 2.4 A comparison of fracture toughness of various HA-based biocomposites. For comparison, plot has been divided into four categories: HA-0x, HA-5x, HA-10x, and HA-20x, where x is the wt% of second phase. HA (w) indicates the use of HA whisker reinforcement and HA-10ZrO₂ (a) indicates the Al₂O₃ coated ZrO₂ particles as second phase. ZrO₂ (f) and ZrO₂ (c) stands for fine grained and coarse grained ZrO₂ particles. The toughness values measured with SEVNB are mentioned and otherwise, those are estimated on the basis of indentation cracking. References related to the respective work are mentioned on individual bar and otherwise, results are from present work. The symbols *, **, #, and ## show the significant difference (measured by Dunnett’s t (2 side) test) between reference sample (from present investigation) and other samples in each category [67]

2.4 Toughening Mechanisms and Toughness Properties

Fracture in brittle solids can be correlated with propagation of crack under tensile loading. Importantly, the crack propagates if and only if the stress at crack front is higher than a critical value. In brittle solids, the resistance against the crack propagation can be increased by introducing the mechanisms that reduce the driving force for the crack propagation by dissipating the additional energy associated with crack tip and therefore leads to the blunting of crack tip [81]. In polycrystalline ceramic materials, the microstructure dependent fracture toughness can be correlated with the dissipation of the crack tip energy and thereby with the reduction in the driving force for the crack propagation [82]. It is generally recognized that weak interfaces, such as grain boundaries and/or matrix-reinforcement boundary are the

preferred crack deflection paths. Moreover, the presence of second phase disturbs the crack propagation by forcing the crack either to go around it or through it. The extent, to which such phenomenon is able to dissipate the energy, depends on the morphology/distribution and mechanical properties of the second phase in ceramic matrix composite [83].

Although the presence of second phase may improve the fracture toughness of composites, the chemical reaction at the interface during processing (e.g. sintering) always offers the challenge for the materials scientists to optimize the toughness. On the basis of microstructural characteristics of the second phase; i.e., nature, shape and size, the toughened composite materials can be categorized as [84]: (a) particulate reinforced composite, (b) whisker reinforced composite, and (c) fiber reinforced composite. In the context of the current review paper, the toughening in metallic particulate reinforced ceramic composites is attributed to the shielding effect to the crack front propagation due to ductile metal reinforcements. In such case, the crack resistance energy of the matrix can be given by [82],

$$R_o = (1 - V_f)2\gamma_B \quad (2.8)$$

where, γ_B is the intrinsic cohesive energy of the matrix material and V_f is the volume fraction of second (reinforcement) phase. Thus, both the matrix as well as the reinforcement material contributes towards the fracture toughness. Therefore, enhanced fracture toughness can be achieved by intelligently designing the composites having mixed shielding mechanisms.

In case of the polycrystalline monolithic ceramic materials, the frontal zone shielding results due to the interaction of the crack front with dislocation cloud and/or microcrack cloud. The activation of the dislocations results in generation of dislocation cloud and this restricts the crack front propagation by applying a stress field on the crack tip [82]. The stress field generated around the dislocation is proportional to the shear modulus (G) and can be given as [85],

$$\sigma \propto \frac{Gb}{r} \quad (2.9)$$

where, G, b, and r are the shear modulus of the material, Burger vector, and distance from the dislocation, respectively. On the other hand, the microcrack cloud can be activated at the stress field of primary cracks at the relatively weaker zone like, grain boundaries and interphase flaws.

Unlike the combination of dislocation as well as microcrack clouds towards the improvement in fracture toughness in brittle ceramics, the phase transformation in zirconia (ZrO_2) can also contribute to the fracture toughness improvement in ZrO_2 reinforced ceramic matrix [82]. In such case, the partially stabilized ZrO_2 in metastable tetragonal state transforms to stable monoclinic phase at the crack tip stress field [81]. This transformation can be controlled by using additives like, MgO , CaO , Y_2O_3 and CeO_2 [81]. In addition, the grain bridging of crack in monolithic polycrystalline materials results in the toughness improvement due to

the dissipation of crack energy as a result of grain sliding and/or fracture. For example, Swanson et al. [86] reported the evolution of grain bridging in pure polycrystalline alumina. In such case, the transgranular fracture may occur in large grains along with primary crack due to high frictional stresses at sliding grain boundary surfaces.

In 1983, Faber and Evans studied the effect of geometrical aspect of second phase on the crack deflection and therefore, effect on fracture toughness [87, 88]. The strength of the interfacial boundary plays an important role in deciding whether a crack will pass through second phase or get deflected. In a case when interface is strong enough to deflect the crack, it penetrates through the second phase. This may result in pinning the advancing crack front at the interaction site. In case of ductile metal phase, this results in dissipation of crack tip energy due to crack bridging.

In the context of biomaterials, the benefits of second phase addition to a ceramic matrix are twofold: (1) second phase improves the fracture toughness dramatically due to frontal wake and/or bridge interface shielding [67] and (2) second phase leads to the modification in surface energy [89], which can be used as a tool to manipulate the cell-material interaction.

Although the reinforcement of ceramic matrix with fiber or whisker results in improvement in fracture toughness, the release of nanosized and needle shaped whisker/fibrous can lead to the severe toxicity to the cells caused by lipid layer damage and genotoxicity [90, 91]. Therefore, the fiber/whisker toughened ceramic composites are not considered for clinical applications, although related toughening mechanisms can be very well adapted to develop HA-based high toughness composites. In parallel, the reinforcement of ceramic matrix with ductile metal particles results in dramatic improvement in mechanical properties due to additional advantage of both ceramic matrix and metal particles. The crack bridging at metal particles results in dissipation of energy associated with crack tip [67, 82]. The fracture energies of pulsed electric current sintered (PECSed) HA–Ti composites, measured by Nakahira et al. [53] using single edge double notched beam method, show a substantial increment with the Ti content. The fracture energies of 9 and 20 J/m² were reported for HA–20 vol.% Ti and HA–25 vol.% Ti samples, respectively. The reason for the improvement in fracture toughness was crack deflection and crack bridging by Ti particles, which are retained in the HA matrix.

Similarly, the significant improvement in the fracture toughness of the spark plasma sintered HA–Ti composites was measured by the present authors (see Fig. 2.8) [67]. An addition of 10 wt% Ti to HA increases the fracture toughness (K_{IC}) from ~ 3.4 to 4.7 MPa m^{1/2}. Further addition of Ti (20 wt%) to HA does not improve the fracture toughness (4.3 MPa m^{1/2}). The increased fracture toughness of HA and HA–Ti composites can be explained on the basis of crack deflection and crack bridging. In the context of toughness enhancement, the synergetic interaction of multiple toughening mechanisms can be explored. The presence of Ti particles provides the significant contribution toward the fracture toughness by crack bridging. Fracture toughness can also be further enhanced with the addition of ZrO₂ particles. The transformation toughening due to ZrO₂ phase along with crack bridging due to Ti phase can therefore provide the synergetic effect in order to

improve the fracture toughness beyond the $4.7 \text{ MPa m}^{1/2}$ (fracture toughness of HA–10 wt% Ti composite). Also, the linear thermal expansion coefficient of ZrO_2 ($10.5 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$) is found to be in between HA ($17.3 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$) and Ti ($8.4 \times 10^{-6} \text{ }^\circ\text{C}^{-1}$). This helps in minimizing the difference in thermal expansion coefficient between HA and Ti and therefore, does not lead to the interfacial debonding in sintered composites.

Several aspects can be discussed to explain the significant increase in the fracture toughness of the HA–Ti composites. It is worthwhile to mention that the reliable measure of toughness properties of HA–Ti has been obtained only using long crack toughness measurement techniques, like SEVNB etc. The most important aspect is that the crack bridging due to lath shaped Ti phase as well as crack wake debonding at HA and Ti interface are responsible for the fracture toughness enhancement ($\sim 4.7 \text{ MPa m}^{1/2}$) in HA–10 wt% Ti. However, no further improvement in fracture toughness of HA–20Ti composites was noted due to non-uniform distribution as well as lower volume fraction of Ti, resulted due to unification of Ti during ball-milling. This can be confirmed using theoretical models proposed by Ashby [92] as well as Evans [93]. The detailed analysis of fracture toughness of particulate (Ti) reinforced ceramic (HA) has been reported elsewhere [67].

According to these models, the particle size and the distribution of the metallic phase near the crack tip (which is directly proportional to the volume fraction of metal phase in the HA matrix) has a strong influence on the fracture toughness. The details are reported elsewhere [92, 93]. Although the shape of metal particles has an effect on fracture toughness, the average number of particles near the crack tip is the deciding factor in the ceramic-metal (e.g. HA–Ti) composites (see Fig. 2.3). The unification of Ti in HA–20Ti composite results in the reduction in volume fraction Ti in HA matrix and therefore reduces the distribution of Ti particles near the crack tip. Therefore, fracture toughness of HA–20 wt% Ti is found to be lower than HA–10 wt% Ti composite.

In future, cryo-milling of the HA–Ti composition may be found as a good alternative to conventional ball-milling in this particular case. The cryo-milling is expected to produce well distributed Ti particles in the starting powder mixture. This will allow well dispersed Ti in HA–Ti composites with concentration of Ti more than 20 wt% causing further improvement in fracture toughness. It may be possible to reduce the initial particle size considerably by cryo-milling. This will lead to the HA–Ti composites with even higher fracture toughness. Therefore, designing the composites with different shape and size of Ti particles may be useful in improving the fracture toughness.

The increased Ti particle distribution by restricting the unification of Ti during ball-milling may provide an opportunity to further increase the fracture toughness. Therefore, cryo-milling can be used in place of conventional high energy ball-milling to restrict the extensive deformation and therefore, unification of Ti particles. The uniform dispersion of metallic reinforcement would also increase the strength properties. In future, cryo-milling parameters need to be optimized and similar SPS experiments can be performed to prove this point.

In last two decades, the fractal modeling approach has been used to a modest extent to predict the fracture toughness of the ceramic composites [94, 95]. Xu and co-workers successfully used the Mandelbrot's modified fractal model to calculate the fracture toughness (K_{IC}) of alumina with equiaxed grains as well as reinforced with elongated grains [96]. According to this model, the microscopic (characterized by irregular crack propagation path) fracture toughness can be given as,

$$K_{IC} = (2E\gamma_s)^{1/2} \left(\frac{1}{r}\right)^{(d_f)/2} \quad (2.10)$$

where, E is the elastic modulus in tension; γ_s is the interfacial energy equals to (Eb_o/π^2) , where, E and b_o are the elastic modulus and atomic dimension, respectively [82]. In Eq. 2.8, d_f is the fractal dimension, which defined as,

$$d_f = \frac{\log(N)}{\log(\frac{1}{r})} \quad (2.11)$$

where, r is the ratio that describe the segments generated in self-similar object. A self-similar object (fractal curve) can be broken into arbitrary small elements with each small element being a replica of the entire object; N is calculated from fractal curve and equal to the number of patterns generated for a ratio ' r ' [97, 98].

Recently, Rishabh et al. [99] have also used the Mandelbrot's modified model to estimate the fracture toughness of monolithic alumina with equiaxed grains. They also calculated the fracture toughness of alumina reinforced with elongated alumina grains as well as with single wall carbon nanotubes. It has been found that the Mandelbrot's modified model can be used to estimate the fracture toughness of ceramic composites instead of using Vickers indentation method. The fractal modeling approach in case of bioceramics is not being extensively utilized to predict the toughness properties and this is one area, which requires attention in future.

In fact, the fracture toughness of monolithic HA ($3.4 \pm 0.2 \text{ MPa m}^{1/2}$) and HA–10Ti ($4.7 \pm 0.1 \text{ MPa m}^{1/2}$) composite reflect on significant improvement compared to earlier literature reports (see Fig. 2.4). In the present case, K_{IC} values are measured by SEVNB method and the toughness values are reproducibly measured on multiple samples.

In Fig. 2.4, the fracture toughness values for HA based biocomposites with 20 % of less reinforcement content are compared with presently investigated materials. Such a comparison would be more effective if we look at the toughness data measured at a constant reinforcement content (e.g. 5, 10, 20 %). Another point to be noted is that indentation cracking method has largely been used to measure the toughness of HA based composites with the exception of the present work and the study by Zhang and co-workers [100]. In view of the dependence of toughness on volume fraction of reinforcement, we only compared our results with the toughness data for those with systematic variation in reinforcement content (≤ 20 %).

As far as monolithic HA is concerned, the present work demonstrates at least three to four times higher toughness than earlier reported values. In case of HA reinforced with 5 and 20 % toughening phases, the present study once again confirms the superiority of spark plasma sintered HA–Ti in terms of exhibiting three to four times higher toughness compared to HA with similar reinforcement content. While similar trend is also observed in case of HA with 10 % reinforcement content, the study by Kong et al. [61] demonstrates that HA, when reinforced with Al₂O₃-coated ZrO₂ (10 %) can exhibit 70 % of the toughness, that we have achieved with the currently developed HA–10 % Ti. Interestingly, the qualitative trend of the toughness increase through a maxima at 10 % reinforcement addition is observed in case of both Ti and ZrO₂ (see Fig. 2.1). A drop in toughness value in both the cases with the addition of larger than 20 % reinforcement therefore rationalizes our effort of not to develop HA composites with 30 % Ti or larger. In the following, high toughness property will now be critically analyzed in the light of the available crack bridging model due to the presence of Ti, as any toughness increment in ceramic composite containing metallic particles must be due to the bridging of crack faces because of the yielding of ductile metallic phase (Ti in the present case) as well as crack deflection.

First of all, the spark plasma sintered monolithic HA exhibits $K_{IC} \approx 3.4 \pm 0.2 \text{ MPa m}^{1/2}$, which is 3 times higher than the reported values in the literature [62, 63, 100–102]. Such an increase in K_{IC} for HA can be related to unique microstructure developed. It is important to mention that the microstructure of HA is characterized by a mixture of submicron equiaxed grains and elongated grains with an average width 1 μm (see Fig. 2.6a). While the major fraction of the grains are equiaxed, a significant fraction is rod shaped grains with aspect ratio of 5 or larger. It is to be noted here that the initial HA particles are of rod shape in nature. This allows the orientation and stacking of HA particles in such a way that results in the increase in the relative intensity corresponding to the (300) plane as compared to the (211) plane. Chaudhry et al. also reported this kind of features in spark plasma sintered nanograined monolithic HA [103].

In order to understand the interaction of a crack path with microstructure, representative SEM images of indented regions on monolithic HA and HA–10Ti composite are shown in Fig. 2.5. Mostly, the montage images are shown to illustrate the crack path over a wider microstructural region. In Fig. 2.5a, the crack emanating from a Vickers indent, taken at 500 gm indent load, is shown and a wavy nature of crack path is visible, as marked by a series of white arrows. Clearly, the crack goes along the grain boundary or deflected/bridged by elongated grains on its path of propagation.

Now, it is worthy to study the effect of Ti addition along with the effect of reaction products (formed at the HA/Ti interface) on the interface debonding as well as mechanical properties. The BSE-SEM images confirm the homogenous distribution of Ti phase in HA matrix (see Fig. 2.5). However, the lath-like Ti phase at higher Ti content results in higher contact area at HA/Ti interface as compared to spherical shaped Ti particles. The observation of irregular or agglomerate like Ti

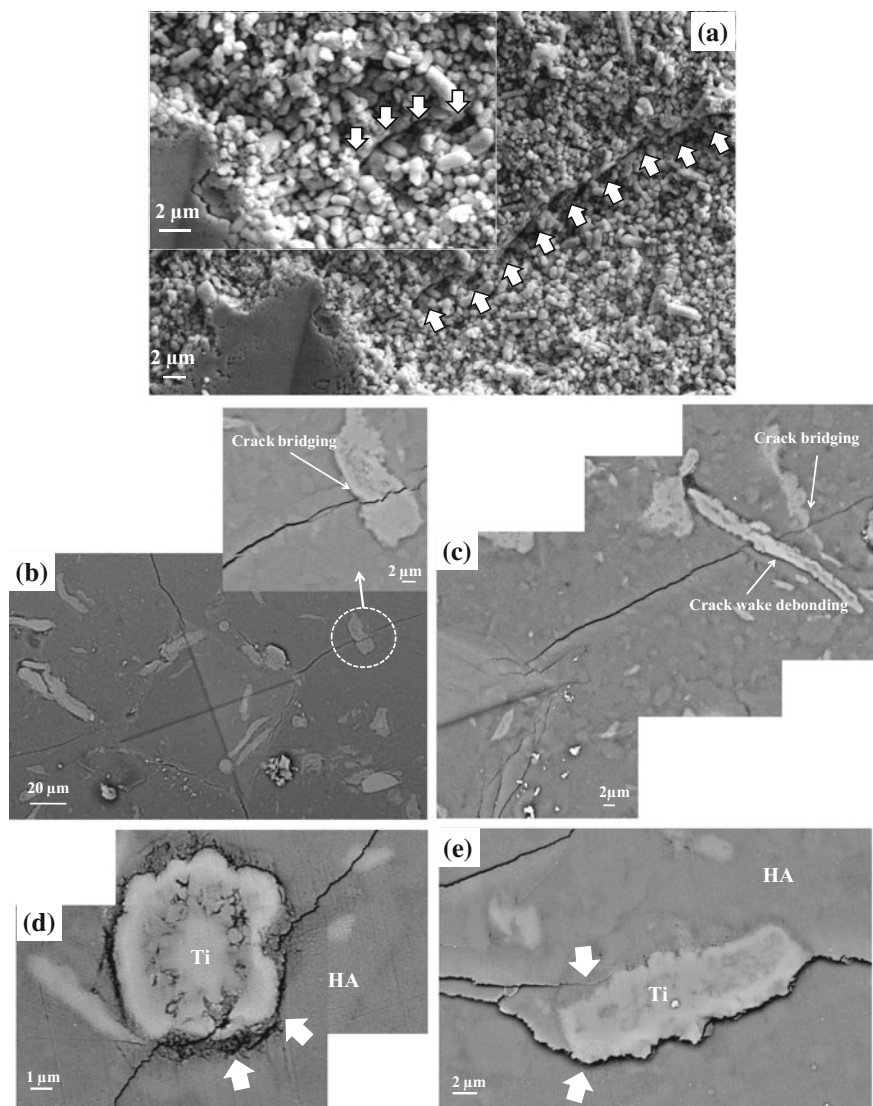


Fig. 2.5 Representative secondary electron SEM micrographs of chemically etched HA (a), showing the indent (500 g load) region and crack path (marked with white arrow). **b** and **c** represent the back scattered electron SEM images of HA–10Ti, showing the indent (at 1000 g load) region and crack path. The crack bridging (marked on 'b' and 'c') by lath shaped/elongated Ti particles and crack wake debonding (marked on 'c') is visible. The crack wake debonding at HA/Ti interface with spherical and lath shaped particles is clearly visible and marked with white arrow on 'd' and 'e', respectively [67]

(bright contrast) in Fig. 2.5b–e indicate either extensive deformation or unification of finer Ti particles during SPS.

BSE-SEM images, shown in Fig. 2.5b, c, provide evidences for passage of crack through Ti-agglomerates as well as crack bridging around lath-shaped Ti phase. Interestingly, these toughening microstructural elements, i.e. Ti phase have an interesting/useful shape with width of 5–10 μm and aspect ratio of 10 or larger. It is worthwhile to mention that such shape of Ti phase is akin to elongated grains in Si_3N_4 -based materials or short whiskers, both of which impart useful toughness. A closer inspection of the inset image of Fig. 2.5b reveals the crack, while going through Ti phase, is being bridged on its original path with apparent decrease in crack face width. In contrast, another crack causes debonding at HA/Ti interface and bridges the crack on the interface (Fig. 2.5c). Significant debond length of over 10 μm could be noticed. More evidences for crack wake debonding at the matrix/particulate interfaces with spherical or lath shaped Ti phase are provided respectively in Fig. 2.5d, e. The crack wake debonding can perhaps be attributed to the presence of Ti and/or $\text{CaTi}_4(\text{PO}_4)_6$ phase and the presence of $\text{CaTi}_4(\text{PO}_4)_6$ was earlier confirmed earlier using TEM analysis. From the above, it can be conclusively stated that both crack deflection and crack bridging contribute towards the toughness enhancement in HA–Ti composites. It can be mentioned that such crack bridging/deflection has also been reported in several ceramic-matrix composites reinforced with whiskers/fibers as well as in case of Ag-reinforced HA [100, 104].

2.5 Correlation with Existing Theoretical Models

In this sub-section, an attempt is being made to correlate the experimentally measured toughness properties with the theoretical model predictions. A theoretical model proposed by Becher et al. [105] was used to estimate the fracture toughness in the present case. It can be noted that Becher's model can successfully explain the toughness of Si_3N_4 -based ceramics with large anisotropic tabular/elongated grains. In such case, fracture toughness can be correlated with microstructure using the following equations [105],

$$K = C \left(\sum_i V_i W_i (AR_i)^2 \right)^{1/2} \quad (2.12)$$

where, V, W and AR represents to the volume fraction of elongated grains, width of elongated grain and aspect ratio of elongated grains, respectively and the suffix denotes the *i*th types of elongated grains.

In the above equation,

$$C = \left(\frac{E\tau}{6(1-\nu^2)} \right)^{1/2} \quad (2.13)$$

where, E (94 GPa), τ (≈ 15 MPa) [106] and ν (≈ 0.3) [107] are the elastic modulus, interfacial shear stress and Poisson's ratio of the elongated grains of HA, respectively. Using above equations and microstructural parameter, the fracture toughness of HA is found to be around ~ 3.2 MPa m^{1/2}, which is very close to the experimentally measured value (~ 3.4 MPa m^{1/2}). In estimating the fracture toughness from the above equation, elastic modulus measured by nanoindentation has been used. This is due to the fact that the indented zone dimension would scale with the interaction length due to crack bridging by elongated grain in monolithic HA.

In the next step, in order to explain the influence of ductile second phase (here Ti) in further improvement of fracture toughness, two different models has been adopted. The first model, proposed by Ashby et al. [92] highlights the effect of volume fraction (F) and particle size (D) of Ti phase on the fracture toughness of HA–Ti composites. The second model, proposed by Evans et al. [93], considers the effect of matrix phase on the fracture toughness of composite. In this model, the fracture toughness of composite strongly depends upon the particle size (D) and distance between near neighboring particles (δ).

Adopting Ashby's model, the increment in the fracture toughness (ΔK_{IC}) in a ceramic matrix composite reinforced with ductile metal particles can be given by the following expression [92],

$$\Delta K_{IC} = (CSFE\sigma_y D)^{1/2} \quad (2.14)$$

where, C is a constant which depends upon the interfacial strength; S is the fraction of particles nearby to the crack tip; F is the volume fraction of the ductile metal particles which equals to the area fraction on the crack plane; E is the elastic modulus of the metallic (Ti) particles (103 GPa) [108]; σ_y is the yield strength of the metallic Ti particles (325 MPa) [109]; D is the size of the Ti particles. The values of F and D were estimated using image processing software (ImageJ) from SEM images captured in BSE mode and some representative images are shown in Fig. 2.5.

Therefore, ΔK_{IC} can be considered to be directly proportional to the $(SFD)^{1/2}$,

$$\Delta K_{IC} \propto (SFD)^{1/2} \quad (2.15a)$$

$$\Delta K_{IC} = C^*(SFD)^{1/2} \quad (2.15b)$$

where, C^* is a constant.

In present case, the volume fraction of Ti in HA–5Ti, HA–10Ti, and HA–20Ti were 0.095, 0.205, and 0.292, as estimated using ImageJ software. The value of S

(the fraction of particles nearby the crack tip) is directly related to the volume fraction of Ti in HA matrix (F). Therefore, S will increase with an increase in F. If we consider the number fraction of particles near the crack tip in case of HA–5Ti i.e. $S = n$, such assumption will lead to, $S \approx 2n$ and $S \approx 3n$ for HA–10Ti and HA–20Ti, respectively.

Based on Eq. (2.16) and above consideration, the increment in fracture toughness varies according to the volume fraction of reinforcement particles and size of the reinforcement particles. In Fig. 2.3a, ΔK_{IC} versus $(SFD)^{1/2}$ for investigated materials is plotted. The results obtained from theoretical model (Fig. 2.2a) shows statistically significant difference between HA–5Ti and HA–10Ti as well as HA–5Ti and HA–20Ti samples, when Dunnett's t (marked with *) test was applied. The Dunnett' C test does not show any significant difference in this case. However, a significant difference between HA and HA–10Ti as well as between HA–5Ti and HA–20Ti samples was found, when Dunnett's t (marked with **) and Dunnett' C (marked with ##) test were used for $(SFD)^{1/2}$. In Fig. 2.2b, the results reveal the significant difference for both $K_{IC(HA-Ti)} - K_{IC(HA)}$ and $(1 + (\delta/D))^{-1/2}$, when Dunnett's t test was applied. However, Dunnett's C test fails to reveal any significant difference in case of $K_{IC(HA-Ti)} - K_{IC(HA)}$ and $(1 + (\delta/D))^{-1/2}$. The strength of HA/Ti interface may lead to the deviation of ΔK_{IC} from its linear pattern. The positive deviation is possibly due to the result of high interfacial strength. In contrast, the negative deviation may be a result of the weak interface.

In order to validate the obtained results from the Ashby's model, we have used another model to estimate the fracture toughness and an attempt has been made to correlate the result obtained with the experimental values (see Fig. 2.3b). The yield strength of the ductile metal particle as well as size and interparticle spacing can also affect the toughness of a ceramic matrix-metal composite, as per the following equation [93],

$$K_{IC} = K_{ICm} + f(u^*, \sigma_y) \frac{1}{\sqrt{1 + \frac{\delta}{D}}} \quad (2.16)$$

where, K_{IC} is the fracture toughness of composite; K_{ICm} is the fracture toughness of ceramic matrix (HA); $f(u^*, \sigma_y)$ is a constant depends upon the metal (Ti) strain to failure (u^*) and the yield strength of the Ti particles (σ_y); δ is the particles near-neighbor distance; D is the size of the Ti particles. In the present case, the values of δ and D were estimated using an image processing software (ImageJ) from SEM images captured in BSE mode (see Fig. 2.5).

For a given ceramic matrix and metal reinforcement, the fracture toughness of composite largely depends on δ and D. For a particular volume fraction of reinforcement, the ratio $(\frac{\delta}{D})$ is independent of particle size and therefore, $(\frac{\delta}{D})$ values do not affect the fracture toughness. However, in case of composite with varying particle size and amount of metal reinforcement, the ratio $(\frac{\delta}{D})$ is highly significant. In the present case, the microstructural analysis shows a lower value of $(\frac{\delta}{D})$ for

HA–20Ti as compared to HA–10Ti. This is due to the unification of Ti particles during ball milling/SPS, which leads to an increase in final particle size.

2.6 Biocompatibility Properties of HA–Ti Composites

The improvement in mechanical properties must not lead to the degradation of biological properties. Although HA and Ti (and Ti alloys) were investigated independently for their good biocompatibility property, only few reports are available on the study of biocompatibility of the HA–Ti composites. In few forthcoming sections, we will discuss briefly the bioactivity, cytocompatibility and biocompatibility of HA–Ti composites processed by advanced sintering route.

The cytotoxicity of a biomaterial can be investigated on the basis of cell viability and proliferation, *in vitro*. Nath et al. [52] reported the results of L929 mouse fibroblast cell adhesion on the conventionally sintered HA–10Ti, HA–20Ti, and HA–30Ti composites. The cultured L929 cells show more flattened morphology on HA–Ti samples (see Fig. 2.6) [52]. In a follow-up work [50], SEM studies reveal the extensive cell-to-cell contact as well as spreading of the Saos-2 cells followed by ECM (extracellular matrix) formation (Fig. 2.7). The well defined morphology of Saos-2 cells on the HA–Ti substrates reveals their viability and establishes the cytocompatibility of the HA–Ti composites. The fluorescence microscopic investigation supports the finding of the SEM study. Extensive actin filament reorganization (green color) and unfragmented nucleus (blue color) confirm the cytocompatibility of the HA–Ti composites (see Fig. 2.7).

2.6.1 *in vivo* Biocompatibility

Although *in vitro* experiments can be used for initial screening of the biomaterials, the absence of actual physiological conditions during these experiments limit the applicability of such experiments. In fact, the material is to be tested for *in vivo* biocompatibility property in order to understand the effect of more diverse environment enriched with enzymes, proteins and variable pH as well as internal and/or external stimuli like stress/electric field. Therefore, *in vivo* experiments are the endpoint study of the biocompatibility of a biomaterial. In order to evaluate the biocompatibility of HA–Ti composites, Chu et al. [71] performed the implantation of HA–20 vol.% Ti in the skull of New Zealand white rabbits. Histological observations after 3 weeks of implantation revealed the partial integration of implant material with host bone. At the beginning of *in vivo* experiment (3 weeks), it has been noticed that the presence of Ti (bioinert) results in a decrease in the

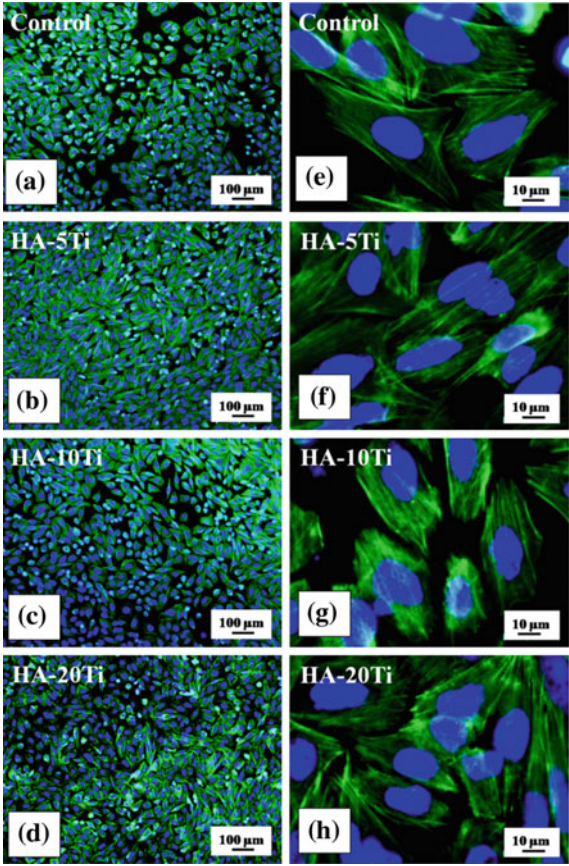


Fig. 2.6 Representative fluorescence microscopy images of SaOS₂ cells on control and different HA–Ti composites [50]

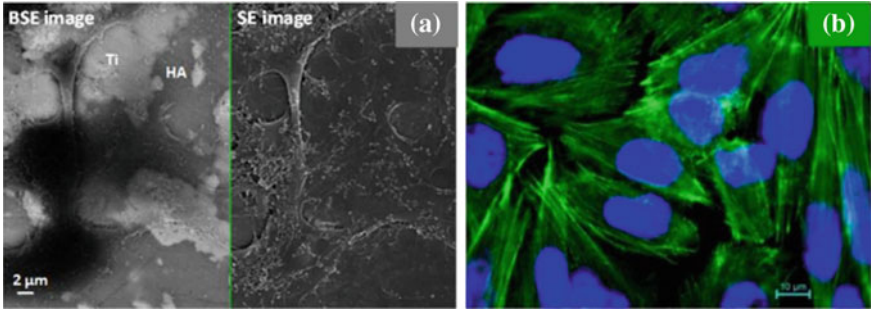


Fig. 2.7 **a** The SEM images showing the cell-to-cell contact and spreading of Saos-2 cells over HA as well as Ti surface of HA–Ti composites. **b** The fluorescence microscopy image of Saos-2 cells, grown on HA–Ti samples shows the extensive reorganization of the actin filaments (green color) and the healthy nuclei (blue color) [50]

osseointegration of the composite. However, after 12 weeks of implantation period, a complete integration of the composite material with host bone was noticed. Therefore, HA–20Ti composite is found to behave similar to HA in terms of long term implantation. Importantly, the decomposition products of HA in HA–20Ti composite such as α -TCP dissolve faster than monolithic HA in the physiological environment and therefore, supply the Ca^{2+} and PO_4^{3-} ions, results in faster healing at the defect site [83].

In a different work, Chu et al. [51] investigated the biocompatibility of HA–Ti FGM (Ti/Ti–20 vol.% HA/Ti–40 vol.% HA), *in vivo*. For this, FGM samples ($3.3 \text{ mm} \times 3.3 \text{ mm} \times 6 \text{ mm}$) were implanted into the skull of New Zealand white rabbits. The bonding strength of FGM was found to be higher than pure Ti at any instant (see Fig. 2.8). It was found that the presence of TiO_2 (rutile) layer on the Ti metal surface provides the nucleation site for the apatite and therefore, enhances the bonding with host tissue [110].

In summary, the presence of HA in the HA–Ti FGM helps in formation of intervening apatite layer and therefore, good integration with host bone. The presence of oxide layer on Ti surface synergistically improves the bioactivity of FGM. Importantly, the addition of Ti to HA does not impair the biocompatibility of the HA–Ti composites.

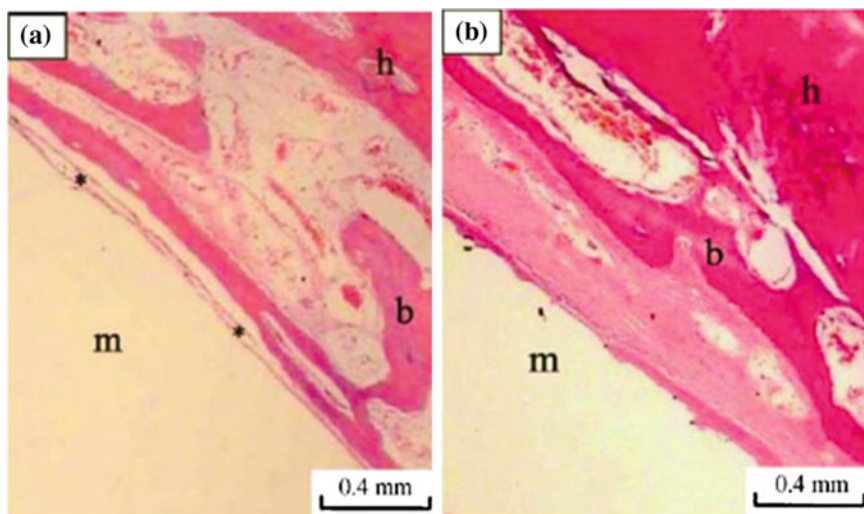


Fig. 2.8 Representative images showing the neobone formation at the interface of implant material **a** Pure Ti and **b** Ti–40 vol.% HA graded layer in the FGM) and host bone, after 4 weeks. Acronyms used: *m* implant, *b* newborn bone, *h* host bone, * fibrous tissue [51]

2.7 Future Perspective

This review summarizes the processing, characterization and properties of HA–Ti based bulk composites in the perspective of bone tissue engineering applications. The processing related challenges in context of phase stability in the HA-based systems are mainly discussed and the shortcomings of the conventional pressureless sintering are highlighted. Such discussion also places the benefits of using advanced processing techniques such as spark plasma sintering (SPS) to consolidate hydroxyapatite–titanium (HA–Ti) composites with maximum retention of HA and Ti in the composite by intelligently tailoring the sintering conditions.

The results of the present study is very significant as with several years of research, the fracture toughness value could not be increased beyond $2 \text{ MPa m}^{1/2}$ even when conventional indentation technique is used and many reinforcements (Al_2O_3 particles, HA whiskers, ZrO_2 particles, silver particles) were attempted [61, 100, 102, 111]. This is the first study showing the promise that the toughness of HA-based materials can be enhanced to $5 \text{ MPa m}^{1/2}$ or beyond. The present study also establishes that Ti addition can be intelligently tailored to obtain a desired combination of fracture toughness, strength and elastic modules. It is possible that synergistic toughening approach with incorporation of ZrO_2 (transformation toughening) can be attempted to further enhance the fracture toughness beyond $5 \text{ MPa m}^{1/2}$. However, this should require a careful control over sintering reactions. To this end, multistage SPS route with two or more intermediate holding at intermediate temperatures can be very effective.

At the closure, the development of high toughness HA biocomposite should have a significant impact on the potential biomedical applications. There has been a great need to develop mechanically reliable HA materials for heavy load bearing implants such as artificial teeth roots [102]. Earlier research reported the *in vivo* biocompatibility as well as clinical acceptance of dense HA as artificial teeth roots [112–114]. It was also reported that most of the loaded implants are broken within one year from implantation because of inferior toughness properties. In the absence of the availability of high toughness materials, dense HA ceramics are used only as unloaded tooth roots substitutes. The present study therefore makes a step ahead in developing more mechanically reliable HA composites for dental restorative applications and other biomedical applications requiring high toughness. Importantly, these high toughness materials do not appear to compromise on the biocompatibility aspects. It is noteworthy to mention that HA–10 % Ti composite, which exhibits highest toughness of close to $5 \text{ MPa m}^{1/2}$ also supports biomineralization kinetics and similar cell viability and Saos-2 cell proliferation *in vitro*, which is either comparable or better than monolithic HA [115]. In our recent study on cell fate process using flow cytometry, it is demonstrated that HA 10 % Ti also supports the progress of cell division of human fetal osteoblast cells without causing any significant necrosis/apoptosis *in vitro* in a statistically significant manner like monolithic HA [116].

References

1. Begley CT, Doherty MJ, Mollan RAB, Wilson JD. Comparative study of the osteoinductive properties of bioceramic, coral and processed bone graft substitutes. *Biomaterials*. 1995;16:1181–5.
2. Silva RV, Camilli JA, Bertran CA, Moreira NH. The use of hydroxyapatite and autogenous cancellous bone grafts to repair bone defects in rats. *Int J Oral Maxillofac Surg*. 2004;10:1–7.
3. Whang PG, Wang JC. Bone graft substitutes for spinal fusion. *Spine J*. 2003;3:155–65.
4. Khan SN, Fraser JF, Sandhu HS, Cammisa FP, Girardi FP, Lane JM. Use of osteopromotive growth factors, demineralized bone matrix and ceramics to enhance spinal fusion. *J Am Acad Orthop Surg*. 2005;13:129–37.
5. Summers BN, Eisenstein SM. Donor site pain from the ilium: a complication of lumbar spine fusion. *J Bone Joint Sur Br*. 1989;71-B:677–680.
6. Giannoudis PV, Dinopoulos H, Tsiridis E. Bone substitutes: an update. *Inj Int J Care Inj*. 2005;365:S20–7.
7. Grauer JN, Beiber JM, Kwon B, Vaccaro AR. The evolution of allograft bone for spinal applications. *Orthopedics*. 2005;28:573–7.
8. Vaccaro AR. The role of the osteoconductive scaffold in synthetic bone graft. *Orthopedics*. 2002;25:571–278.
9. Bonfiglio M, Jeter WS. Immunological responses to bone. *Clin Orthop*. 1972;18:19–27.
10. GD GDB, Goldberg VM, Zika JM. Immune responses of rats to frozen bone allografts. *J Bone Joint Sur, Am*. 1983;65-A:239–246.
11. Damien CJ, Parsons JR. Bone graft and bone graft substitutes: a review of current technology and applications. *J Appl Biomater*. 1991;2:187–208.
12. Pelker RR, Friedlaender GE. Biomechanical aspects of bone autografts and allografts. *Orthop Clin North Am*. 1987;18:235–9.
13. Goldberg VM, Stevenson S, Shaffer JW. *Biology of autografts and allografts*. Park Ridge, Illinois: The American Academy of Orthopaedic Surgeons; 1991.
14. Wiles P. The surgery of the osteo-arthritic hip. *Br j Sur*. 1958;45(193):488–97.
15. Williams DF. On the nature of biomaterials. *Biomaterials*. 2009;30:5897–909.
16. Basu B, Katti DS, Kumar A. *Advanced biomaterials: fundamentals, processing and applications*. New Jersey, USA: John Wiley & Sons, Inc.; 2009.
17. Valiathan M, Krishnan V. *Biomaterials: an overview*. *Natl Med J India*. 1999;12:270–344.
18. LeGeros RZ, Daculsi G, LeGeros JP. *Bioactive bioceramics*. Totowa, NJ: Humana Press; 2008.
19. Hench LL, Paschall HA. Direct bonding of bioactive glass ceramics to bone and muscle. *J Biomed Mater Res*. 1973;4:25–42.
20. Hench LL, Wilson J. Surface-active biomaterials. *Science*. 1984;226:630–6.
21. Osborn JF, Newesely H. The material science of calcium phosphate ceramics. *Biomaterials*. 1980;1:108–11.
22. Legeros RZ, Craig RG. Strategies to affect bone remodeling: osteointegration. *J Bone Miner Res*. 1993;8(S2):S583–96.
23. LeGeros RZ. Properties of osteoconductive biomaterials: calcium phosphate. *Clin Orthop Relat Res*. 2002;395:81–98.
24. Oonishi H, Hench LL, Wilson J, Sugihara F. Quantitative comparison of bone growth behaviour in granules of Bioglass[®], A-W glass-ceramic, and hydroxyapatite. *J Biomed Mater Res*. 2000;51:37–46.
25. Urist MR, Silverman BF, Buring K, Dubuc FI, Rosenberg J. The bone induction principle. *Clin Orthop*. 1967;53:243–83.
26. Geetha M, Singh AK, Asokamani R, Gogia AK. Ti based biomaterials, the ultimate choice for orthopaedic implants-A review. *Prog Mater Sci*. 2009;54:397–425.
27. Morais JM, Papadimitrakopoulos F, Burgers DJ. Biomaterials/tissue interactions: possible solutions to overcome foreign body response. *Am Assoc Pharmaceutical Sci J*. 2010;12:188–96.

28. Alliot-Licht B, Gregoire M, Orly I, Menanteau J. Cellular activity of osteoblasts in the presence of hydroxyapatite: an *in vitro* experiment. *Biomaterials*. 1991;12:752–6.
29. Katti KS. Biomaterials in total joint replacement. *Colloids Surf, B*. 2004;39:133–42.
30. Biring G, Banks M, Meswania J, Blunn G. Friction, lubrication, wear and corrosion. Great Britain: Hodder Arnold; 2007. p. 304.
31. Sansone V, Pagani D, Melato M. The effects on bone cells of metal ions released from orthopaedic implants. A review. *Clinical cases in mineral and bone. Metabolism*. 2013;10:34–40.
32. Kienapfel H, Sprey C, Wilke A, Griss R. Implant fixation by bone ingrowth. *J Arthroplasty*. 1999;14:355–68.
33. Niinomi M. Recent metallic materials for biomedical applications. *Metall Mater Trans A*. 2002;33A:477–86.
34. Saenz A, Brostow W, Rivera E, Castaño V. Ceramic biomaterials: an introductory overview. *J Mater Educ*. 1999;21:297–306.
35. Rivera-Muñoz E, Díaz J, Rodríguez JR, Brostow W, Castaño V. Hydroxyapatite spheres with controlled porosity for eye ball prosthesis: processing and characterization. *J Mater Sci Mater Med*. 2001;12:305–16.
36. Park J. Bioceramics: properties, characterizations, and applications. New York: Springer; 2008. p. 359.
37. Wolff J. The law of bone remodelling, Translated by P.Maquet and R. Furlong. Heidelberg: Springer; 1986.
38. Prendergast PJ, Huiskes R. The biomechanics of Wolff's law: recent advances. *Ir J Med Sci*. 1995;164:152–4.
39. Dujovne AR, Bobyn JD, Krygier JJ, Miller JE, Brooks CE. Mechanical compatibility of noncemented hip prostheses with the human femur. *J Arthroplast*. 1993;8:7–22.
40. Engh CA, Bobyn JD. The influence of stem size and extent of porous coating on femoral bone resorption after primary cementless hip arthroplasty. *Clin Orthop Relat Res*. 1988;231:7–28.
41. Dowson D. Bio-tribology of natural and replacement synovial joints. New York: Springer; 1992.
42. Murugan R, Ramakrishna S. Nanostructured biomaterials. California: American Scientific Publishers; 2004.
43. Hench LL. Bioceramics. *J Am Ceram Soc*. 1998;81:1705–28.
44. Audekercke RV, Martens M, Hastings GW. Mechanical properties of cancellous bone. Florida: CRC Press-Boca Raton; 1984.
45. Black J. Orthopedic biomaterials in research and practice. New York: Churchill Livingston; 1988.
46. Jee WSS. Histology, cell and tissue biology. New York: Elsevier Science Inc.; 1983.
47. Evans FG, King A. Biomedical studies of the musculoskeletal system. Illinois: Springfield; 1961.
48. Bonfield W, Hastings GW. Elasticity and viscoelasticity of cortical bone. Florida: CRC Press-Boca Raton; 1984.
49. Kumar A, Webster TJ, Biswas K, Basu B. Flow cytometry analysis of human fetal osteoblast fate processes on spark plasma sintered Hydroxyapatite-Titanium biocomposites. *J Biomed Mater Res, Part A*. 2013;101:2925–38.
50. Kumar A, Dhara S, Biswas K, Basu B. In vitro bioactivity and cytocompatibility properties of spark plasma sintered HA–Ti composites. *J Biomed Mater Res Part B-Appl Biomater*. 2013;101B:223–36.
51. Chu C, Xue X, Zhu J, Yin Z. In vivo study on biocompatibility and bonding strength of Ti/Ti-20 vol.% HA/Ti-40 vol.% HA functionally graded biomaterial with bone tissue in the rabbit. *Mater Sci Eng, A*. 2006;429:18–24.
52. Nath S, Tripathi R, Basu B. Understanding phase stability, microstructure development and biocompatibility in calcium phosphate–titania composites, synthesized from hydroxyapatite and titanium powder mix. *Mater Sci Eng, C*. 2009;29:97–107.

53. Nakahira A, Eguchi K. Evaluation of microstructure and some properties of hydroxyapatite/Ti composites. *J Ceram Process Res.* 2001;2:108–12.
54. Ning CQ, Zhou Y. In vitro bioactivity of a biocomposite fabricated from HA and Ti powder metallurgy method. *Biomaterials.* 2002;23:2909–15.
55. Yang Y, Kim K-H, Agrawal CM, Ong JL. Interaction of hydroxyapatite–titanium at elevated temperature in vacuum environment. *Biomaterials.* 2004;25:2927–32.
56. Dubey AK, A AE, Balani K, Basu B. Multifunctional properties of multi-stage spark plasma sintered HA-BaTiO₃ based piezobiocomposites for bone replacement applications. *J Am Ceram Soc.* 2013.
57. Mallik PK, Basu B. Better early osteogenesis of electroconductive hydroxyapatite–calcium titanate composites in a rabbit animal model. *J Biomed Mater Res Part A.* 2013:n/a-n/a.
58. Bajpai I, Balani K, Basu B. Spark plasma sintered HA-Fe₃O₄-based multifunctional magnetic biocomposites. *J Am Ceram Soc.* 2013;96(7):2100–8.
59. Saha N, Dubey AK, Basu B. Cellular proliferation, cellular viability, and biocompatibility of HA-ZnO composites. *J Biomed Mater Res B Appl Biomater.* 2012;100B(1):256–64.
60. Mandal S, Sunilkumar B, Kumar A, Basu B. Hot pressed silver doped hydroxyapatite biomaterials with bactericidal properties against magnetotactic bacteria. *Mater Technol Adv Perform Mater.* 2013.
61. Kong Y-M, Kim S, Kim H-E. Reinforcement of hydroxyapatite bioceramic by addition of ZrO₂ Coated with Al₂O₃. *J Am Ceram Soc.* 1999;82:2963.
62. Rapacz-Kmita A, Siószarczyk A, Paszkiewicz Z. Mechanical properties of HAP–ZrO₂ composites. *J Eur Ceram Soc.* 2006;26:1481–8.
63. Chun Y, Ying-kui G, Mi-lin Z. Thermal decomposition and mechanical properties of hydroxyapatite ceramic. *Trans Nonferrous Met Soc China.* 2010;20:254–8.
64. Long M, Rack HJ. Titanium alloys in total joint replacement—a materials science perspective: Review. *Biomaterials.* 1998;19:1621–39.
65. Murugan R, Ramakrishna S. Development of nanocomposites for bone grafting. *Compos Sci Technol.* 2005;65:2385–406.
66. Kwon S-H, Jun Y-K, Hong S-H, Kim H-E. Synthesis and dissolution behavior of Beta-TCP and HA/Beta-TCP composite powders. *J Eur Ceram Soc.* 2003;23:1039–45.
67. Kumar A, Biswas K, Basu B. On the toughness enhancement in hydroxyapatite-based composites. *Acta Mater.* 2013;61(14):5198–215.
68. Reddy KM, Kumar N, Basu B. Innovative multi-stage spark plasma sintering to obtain strong and tough ultrafine-grained ceramics. *Scripta Materialia.* 2010;62:435–8.
69. Fahami A, Ebrahimi-Kahrizsangi R, Nasiri-Tabrizi B. Mechanochemical synthesis of hydroxyapatite/titanium nanocomposite. *Solid State Sci.* 2011;13:135–41.
70. Sanosh KP, Chu M-C, Balakrishnan A, Lee Y-J, Kim TN, Cho S-J. Synthesis of nano hydroxyapatite powder that simulate teeth particle morphology and composition. *Curr Appl Phys.* 2009;9(6):1459–62.
71. Chu C, Lin P, Dong Y, Xue X, Zhu J, Yin Z. Fabrication and characterization of hydroxyapatite reinforced with 20 vol%Ti particles for use as hard tissue replacement. *J Mater Sci Mater Med.* 2002;13:985–92.
72. Babushkin O, Lindbäck T, Holmgren A, Li J, Hermansson L. Thermal expansion of hot isostatically pressed hydroxyapatite. *J Mater Chem.* 1994;4:413–5.
73. Lütjering G, Williams JC. *Titanium.* New York: Springer; 2007. p. 442.
74. Weng J, Liu X, Zhang X, Ji X. Thermal decomposition of hydroxyapatite structure induced by titanium and its oxides. *J Mater Sci Lett.* 1994;13:159–61.
75. Groot Kd. *Bioceramics of calcium phosphate.* Boca Raton, Florida: CRC Press; 1983.
76. Ning CQ, Zhou Y. In vitro bioactivity of a biocomposite fabricated from HA and Ti powder by powder metallurgy method. *Biomaterials.* 2002;23:2909–15.
77. Mondal D, Nguyen L, Oh I-H, Lee B-T. Microstructure and biocompatibility of composite biomaterials fabricated from titanium and tricalcium phosphate by spark plasma sintering. *J Biomed Mater Res, Part A.* 2013;101A(5):1489–501.

78. Beegan D, Chowdhury S, Laugier MT. Work of indentation methods for determining copper film hardness. *Surf Coat Technol.* 2005;192:57–63.
79. Kumar A, Dhara S, Biswas K, Basu B. In vitro bioactivity and cytocompatibility properties of spark plasma sintered HA–Ti composites. *J Biomed Mater Res: Part B-Appl Biomater.* 2012.
80. Zioupos P, Currey JD. Changes in the stiffness, strength, and toughness of human cortical bone with age. *Bone.* 1998;22(1):57–66.
81. Basu B, Balani K. *Advanced structural ceramics.* New Jersey, USA: John Wiley & Sons; 2011. p. 474.
82. Lawn B. *Fracture of brittle solids.* Cambridge, UK: Cambridge University Press; 1998. p. 378.
83. Gang B, Chung-Yuen H. Effects of interface debonding on the toughness of ductile-particle reinforced ceramics. *Int J Solids Struct.* 1990;26(5–6):631–42.
84. Kaw AK. *Mechanics of composite materials.* Boca Raton, Florida, USA: CRC Press; 2006. p. 466.
85. Weertman J. *Dislocation based fracture mechanics.* Singapore: World Sci; 1996.
86. Swanson PL. *Crack-interface traction: a fracture-resistance mechanism in brittle polycrystals.* Westerville, OH: American Ceramic Society; 1988.
87. Faber KT, Evans AG. Crack deflection processes—I. Theory. *Acta Metall.* 1983;31(4):565–76.
88. Faber KT, Evans AG. Crack deflection processes—II. Experiment. *Acta Metall.* 1983;31(4):577–84.
89. Kumar A. *Microstructure development, biomineralization and osteoblast cell fate processes on high toughness hydroxyapatite-titanium composites.* Kanpur, India: Indian Inst Technol Kanpur; 2013. p. 204.
90. Li Y, Yuan H, von dem Bussche A, Creighton M, Hurt RH, Kane AB, Gao H. Graphene microsheets enter cells through spontaneous membrane penetration at edge asperities and corner sites. *Proceedings of the National Academy of Sciences;* 2013.
91. Billi F, Campbell P. Nanotoxicology of metal wear particles in total joint arthroplasty: a review of current concepts. *J Appl Biomater Biomech.* 2010;8(1):1–6.
92. Ashby MF, Blunt FJ, Bannister M. Flow characteristics of highly constrained metal wires. *Acta Metall.* 1989;37(7):1847–57.
93. Evans AG, McMeeking RM. On the toughening of ceramics by strong reinforcements. *Acta Metall.* 1986;34:2435.
94. Shou-Zhu Z, Chi-Wei L. Fractal dimension and fracture toughness. *J Phys D Appl Phys.* 1989;22(6):790.
95. Mandelbrot BB, Passoja DE, Paullay AJ. Fractal character of fracture surfaces of metals. *Nature.* 1984;308:721–2.
96. Xu L, Xie Z, Gao L, Wang X, Lian F, Liu T, Li W. Synthesis, evaluation and characterization of alumina ceramics with elongated grains. *Ceram Int.* 2005;31(7):953–8.
97. Charkaluk E, Biggerelle M, Iost A. Fractals and fracture. *Eng Fract Mech.* 1998;61(1):119–39.
98. Carpinteri A. Fractal nature of material microstructure and size effects on apparent mechanical properties. *Mech Mater.* 1994;18(2):89–101.
99. Rishabh A, Joshi MR, Balani K. Fractal model for estimating fracture toughness of carbon nanotube reinforced aluminum oxide. *J Appl Phys.* 2010;107:7.
100. Zhang X, Gubbels GHM, Terpstra RA, Metselaar R. Toughening of calcium hydroxyapatite with silver particles. *J Mater Sci.* 1997;32(1):235–43.
101. Tancred DC, McCormack BAO, Carr AJ. A quantitative study of the sintering and mechanical properties of hydroxyapatite/phosphate glass composites. *Biomaterials.* 1998;19:1735–43.
102. Suchanek W, Yashima M, Kakihana M, Yoshimura M. Hydroxyapatite/hydroxyapatite-whisker composites without sintering additives: mechanical properties and microstructural evolution. *J Am Ceram Soc.* 1997;80:2805–13.

103. Chaudhry AA, Yan H, Gong K, Inam F, Viola G, Reece MJ, Goodall JBM, ur Rehman I, McNeil-Watson FK, Corbett JCW and others. High-strength nanograined and translucent hydroxyapatite monoliths via continuous hydrothermal synthesis and optimized spark plasma sintering. *Acta Biomater.* 2011;7(2):791–799.
104. Warren R. Fundamental aspects of the properties of ceramic-matrix composites. London, UK: Blakie and Sons Ltd.; 1992.
105. Becher PF, Lin HT, Hwang SL, Hoffmann MJ, Chen I-W. The Influence of microstructure on the mechanical behavior of silicon nitride ceramics. In: *MRS online proceedings library* 1992;287:null-null.
106. Wei M, Ruys AJ, Swain MV, Kim SH, Milthorpe BK, Sorrell CC. Interfacial bond strength of electrophoretically deposited hydroxyapatite coatings on metals. *J Mater Sci Mater Med.* 1999;10(7):401–9.
107. Prokopiev O, Sevostianov I. Dependence of the mechanical properties of sintered hydroxyapatite on the sintering temperature. *Mater Sci Eng. A.* 2006;431:218–27.
108. Niinomi M. Mechanical properties of biomedical titanium alloys. *Mater Sci Eng. A.* 1998;243:231–6.
109. Zadra M, Casari F, Girardini L, Molinari A. Microstructure and mechanical properties of cp-titanium produced by spark plasma sintering. *Powder Metall.* 2008;51:59–65.
110. Ghaith ES, Hayakawa T, Kasuga T, Nogami M. Apatite formation on rutile type TiO₂ films formed by laser irradiation. *J Mater Sci.* 2006;41(8):2521–4.
111. Kumar R, Prakash KH, Cheang P, Khor KA. Microstructure and mechanical properties of spark plasma sintered zirconia-hydroxyapatite nano-composite powders. *Acta Mater.* 2005;53:2327–35.
112. Ogiso M, Tabata T, Ichijo T, Borgese D. Examination of human bone surrounded by a dense hydroxyapatite dental implant after long-term use. *J Long Term Eff Med Implant.* 1992;2:235–47.
113. Denissen HW, Kalk W, Veldhuis AAH, van den Hooff A. Eleven-year study of hydroxyapatite implants. *J Prosthet Dent.* 1989;61(6):706–12.
114. de Putter C, de Groot K, Smitt PAES. Transmucosal implants of dense hydroxylapatite. *J Prosthet Dent.* 1983;49(1):87–95.
115. Kumar A, Dhara S, Biswas K, Basu B. In vitro bioactivity and cytocompatibility properties of spark plasma sintered HA–Ti composites. *J Biomed Mater Res B Appl Biomater.* 2013;101B(2):223–36.
116. Kumar A, Webster TJ, Biswas K, Basu B. Flow cytometry analysis of human fetal osteoblast fate processes on spark plasma sintered Hydroxyapatite–Titanium biocomposites. *J Biomed Mater Res, Part A.* 2013;. doi:[10.1002/jbm.a.34603](https://doi.org/10.1002/jbm.a.34603).

Biomaterials for Musculoskeletal Regeneration
Applications

Basu, B.; Ghosh, S.

2017, XXXII, 262 p. 94 illus., 21 illus. in color., Hardcover

ISBN: 978-981-10-3016-1