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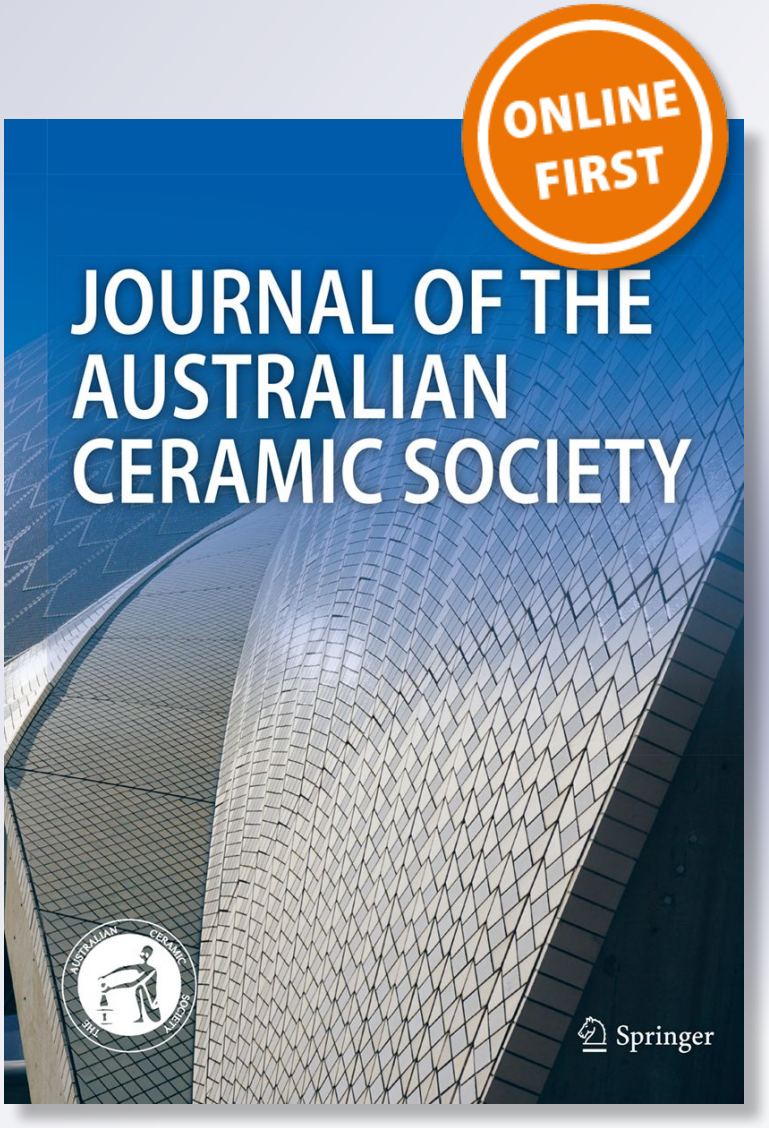

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Preparation of HA/β-TCP scaffold and mechanical strength optimization using a genetic algorithm method

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Abstract Calcium phosphate is widely used as bone scaffold because of its degradation and biocompatible properties. The scaffold must provide high surface area for cellular adhesion from biological surrounding, and suitable mechanical properties for the substitution of damaged bones. Also, the scaffold must be biocompatible, degradable, and the resorption controlled to match those of natural tissues. There are numerous procedures designed for the scaffold that depend on innovation and experiment. The scaffold was prepared by sacrificial method of mixing calcium phosphate powder with polymeric powder polyethylene glycol (PEG). Then, pressing in the cylindrical mold uniaxially, and porous structure of hydroxyapatite and β-tricalcium phosphate (HA/β-TCP) was obtained after sintering. Genetic algorithm optimization was used to optimize the compressive strength; the ratio of brushite and porosity were the parameters of problem. By maximizing the objective function genetic optimization showed that the best compressive strength was (54.67MPa) at 25 wt% ratio of brushite and 40% volume fraction of porosity and experimental value (53.3MPa).The slight difference between these values, perhaps attributable to uncontrolled conditions such as moisture, presence of agglomerate, and the morphology of porosity and other factors, could have led to this difference.

Keywords Calcium phosphate · Genetic algorithm · Scaffold · Mechanical strength · Hydroxyapatite

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Introduction

Reconstructive surgery, repair, and regeneration of defective bone present major challenges. The use of autograft (from the patient) and allograft (from donor) are associated with multiple complication and risks. This has resulted in worldwide attempts for the development of an artificial bone substitute [1]. Therefore, the development of interconnected porous scaffold plays a significant role in bone tissue engineering [2]. For this reason, there are several characteristics that are considered to be essential for the bone scaffold, such as bio-compatibility, osteo-conductivity, and osteo-inductivity. The osteo-conductive scaffold permits the attachment of cells, growth, and extracellular matrix formation of bone related cells on its surface. The osteo-inductive scaffold can actively induce new bone formation. The scaffold also should be porous with interconnected porosity to mimic the architecture of the bone and have suitable mechanical properties, such as being biodegradable at a controlled rate that matches the rate of bone formation [3]. Processing routes for macroporous ceramic are classified into replica method, sacrificial template, and direct foaming method. There are other procedures that are used to prepare ceramic scaffolds, such as freeze casting [4], electrospinning [5], fiber bonding, and poregen leaching [6]. The modern procedure for the preparation of tissues and scaffold are rapid prototyping technologies [7]. Hence, calcium phosphates (CP) have been used in the field of biomaterials as bone graft substitutes. This is because these materials have clinical application in orthopedic, spinal, and maxillofacial surgery. These materials have been regarded as bioactive and osteo-conductive, bonding directly to bone tissue without an interfacial fibrous tissue layer. Additionally, their bioactivity is related to the solubility of (CP) in physiological media [8]. The most

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important parameters at calcium orthophosphate are the molar Ca/P ratio, basicity/acidity, and solubility. Consequently, the lower Ca/P molar ratio is more acidic and water soluble, (CP) with Ca/P ratio less than 1 are not suitable for biological implantation. But, if the ratio is higher than 1.67 the resorption rate dramatically decreases [9], consequently, the genetic algorithm (GA) developed by John Holland and his collaborators in the 1975. Accordance with that, the GA based on principle of natural genetics and natural selection and the strongest specimens will survive. As a result, GA is regarded as one of the most successful meta-heuristic techniques for solving combinatorial optimization problems [10]. Thus, a system using GA produces a variety of (individuals) and selects the fittest according to criteria, change the individual, and repeat the process on the next generation [11]. In accordance with that, GA has been applied to a wide range of optimization problems from graph coloring to pattern recognition. While, the discrete systems such as the traveling salesman problem to continuous system such as the efficient design of airfoil in aerospace engineering. Also, the financial market to multi-objective engineering optimization [12]. However, the GA differs substantially from more traditional search and optimization methods. So, the four most significant differences are: GA search a population of the points in parallel, it is not a single point, GA does not require derivative information or another auxiliary knowledge; it is only the objective function and corresponding fitness levels that influence the directions of search, GA uses probabilistic transition rules, is not deterministic ones, GA works on an encoding of the parameter set rather than the parameter set itself (except where real-valued individuals are used).

Method and Procedures

HA/β-TCP Preparation

HA/β-TCP was produced by solid state reaction by mixing different weight percent ratios (20, 25, 30, 35, and 40wt%) of brushite with HA derived from bovine bone and calcined at 700 °C by ball-milling mixing for 1 h. The mixture was heated in a crucible at 1000 °C for 3 h in a furnace (Protherm, Turkey, Department of ceramics Engineering and Building Materials, University of Babylon). Brushite is first decomposed to monetite at 180 °C and then decomposed to calcium pyrophosphate (Ca2P2O7) at 340 °C, which converts to β phase at 700 °C according to the following equations:

$$CaHPO_4.2H_2O \rightarrow CaHPO_4 + 2H_2O \quad (1)$$

$$2CaHPO_4 \rightarrow Ca_2P_2O_7 + H_2O \quad (2)$$

The obtained Ca2P2O7 reacts entirely with HA to produce mixture of HA/β-TCP in different ratios according to the following equation:

$$Ca_2P_2O_7 + Ca_{10}(PO_4)_6(OH)_2 \rightarrow 4Ca_3(PO_4)_2 + H_2O \quad (3)$$

Scaffold Preparation

The scaffold was prepared by mixing calcium phosphate powders with powder of PEG; its molecular weight 4000 g/mol (BDH, England, 99%purity) with diameter range (100–1000 μm) as poregen at different weight percent ratios (3%, 5%, 7%, and 10%). The study has added 2% polyvinyl alcohol (PVA), (SCR, China with 99% purity) to bind the mixture and wet PEG powder, increase weight present of PEG over 10% lead to crushing the samples. Table 1 shows the calcium phosphate with PEG in different mixing ratios, and formed using die pressing method by compacting the powder in steel die of diameter 16.3 mm by compression hydraulic device at compaction pressure (200 MPa) in loading rate (0.5KN/sec). The samples are then dried by heating at 500 °C for 2 h to remove all the organic material and sintering in 1000 °C within 1 h at a rate 16.6 °C/min. until it reaches 1000 °C. The temperature is maintained for 2 h, then cooled down in furnace to room temperature. The sintering program is shown in Fig 1.

Porosity of Samples

The specimens were tested by Archimedes method according to (ASTM C373-88). The apparent porosity is relationship of volume of open pores to exterior volume and calculated as follows:

$$\boldsymbol{P} = \left(\frac{\boldsymbol{M}-\boldsymbol{D}}{\boldsymbol{V}}\right) * 100 \quad (4)$$

We can calculate the total porosity (P_t^t) by the following equation:

$$\boldsymbol{P_t} = 1 - \left(\frac{\rho_b{}^{\boldsymbol{b}}}{\rho_{th}{}^{\boldsymbol{th}}}\right) \quad (5)$$

where $P_b{}^b$ is bulk density, $P_{th}{}^{th}$ is theoretical density of volume mixture of β-TCP and HA. The theoretical density of β-TCP is 3.07g.cm^{-}3 [13] whereas the density of HA is 3.156 g.cm^{-}3[14].

Bulk Density

The bulk density of specimens is calculated after sintering according to ASTM (373-88) by the following equation:

Table 1 Weight percentage of calcium phosphate and PEG at each experiment

Experiments no.	Calcium phosphate powder (wt%)	PEG(wt%)
1.	(80%HA,20%brushite)100%	0%
2.	(80%HA,20%brushite) 97%	3%
3.	(80%HA,20%brushite) 95%	5%
4.	(80%HA,20%brushite) 93%	7%
5.	(80%HA,20%brushite)90%	10%
6.	(75%HA,25%brushite)100%	0%
7.	(75%HA,25%brushite)97%	3%
8.	(75%HA,25%brushite)95%	5%
9.	(75%HA,25%brushite)93%	7%
10.	(75%HA,25%brushite)90%	10%
11.	(70%HA,30%brushite)100%	0%
12.	(70%HA,30%brushite) 97%	3%
13.	(70%HA,30%brushite) 95%	5%
14.	(70%HA,30%brushite) 93%	7%
15.	(70%HA,30%brushite)90%	10%
16.	(65%HA,35%brushite)100%	0%
17.	(65%HA,35%brushite) 97%	3%
18.	(65%HA,35%brushite) 95%	5%
19.	(65%HA,35%brushite) 93%	7%
20.	(65%HA,35%brushite)90%	10%
21.	(60%HA,40%brushite)100%	0%
22.	(60%HA,40%brushite) 97%	3%
23.	(60%HA,40%brushite) 95%	5%
24.	(60%HA,40%brushite) 93%	7%
25.	(60%HA,40%brushite)90%	10%

$$\rho_b{}^{b} = D \Big/ V \tag{6}$$

Mechanical Testing for Scaffold

Compressive Strength

The samples were prepared according to (ASTM C773-88); the prepared specimen is cylindrical and the diameter is (16.3 mm), the compressive force calculating using (the hydraulic universal material tester, 50 KN load cell) with compressive rate (0.3 KN/s), the compressive strength determined by using the following equation:

$$\sigma = \frac{P}{A} \tag{6}$$

The mean compressive strength is taken for each mixture.

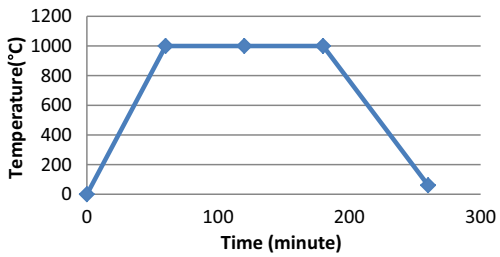

Fig. 1 the program of sintering

Result and Discussion

XRD Results

The phases of prepared powders were identify using XRD (Shimadzu 6000, Japan, Department of Ceramics Engineering and Building Materials, University of Babylon) in room temperature using Cukα radiation. The X-ray wavelength was (λ = 1.5405 Å) at an incident angle (θ), with scanning speed of 5°/min and an applied power of 40 kv/30 mA. Figure 2a, b show the XRD results of (a) (HA/brushite) mixture before, and (b) after heat treatment at 1000 °C at 20% brushite ratio, respectively. The appearance of β-TCP peak refers to that reaction that occurred between HA and (Ca2P2O7). This is formed because of the decomposition of brushite in two steps to form Ca2P2O7. For that reason, the peak for Ca2P2O7 after heat treatment not exit and this indicates that Ca2P2O7 completely reacting with part of HA to form HA/β-TCP mixture. The results have shown that increase in the brushite ratio leads to increase in the β-TCP ratio at the expense of hydroxyapatite ratio due to increase in Ca2P2O7 that was obtained from decomposition of brushite phase.

Physical and Mechanical Results

At 20% brushite ratio addition the apparent porosity 34.11vol% for samples without the addition of PEG, the research has shown that porosity increases from 41.9 to 44.7 vol % with increase in the PEG ratio from 3 to 10%, Table 2. This indicates that most of the polymer addition was removed with heating process. Table 2 also shows the same behavior in increasing the porosity with increase in brushite ratio from 20 to 40 wt%. Additionally, the research has observed that there is a slight increase in porosity with increase brushite ratio from 20 to 40 wt% without the addition of PEG; this is because of the decomposition of brushite.

On the other hand, the compressive strength changes with variation in volume fraction of porosity and HA/β-TCP ratio, and also is affected by pore size, pore morphology, and interconnectivity of pores. In Fig. 3, relation between compressive strength and brushite addition is shown. There is an increase in compressive strength from 28.41 to 53.3MPa with increase in

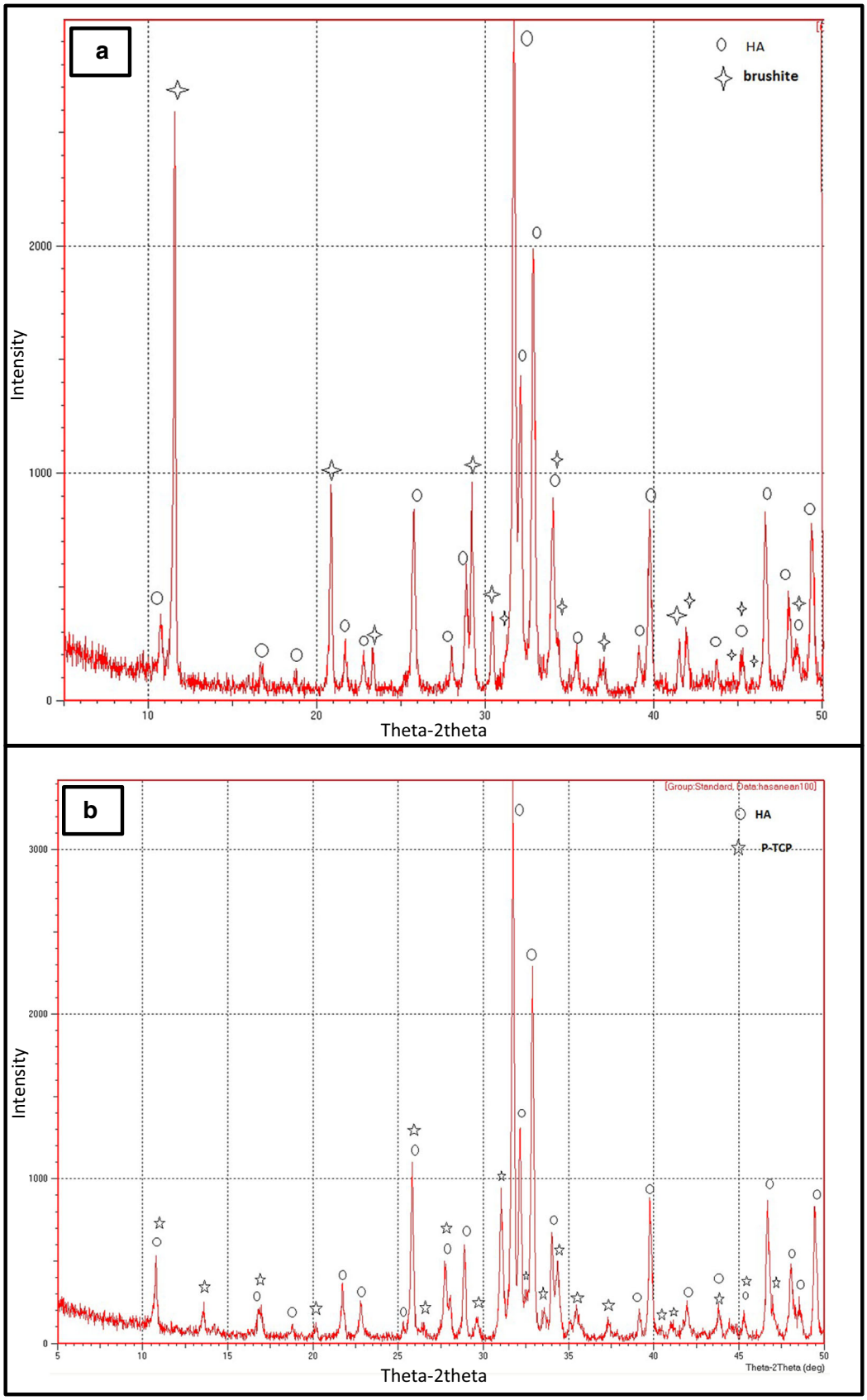

Fig. 2 XRD pattern of **(a)** (HA/brushite) mixture before heat treatment; **(b)** (HA/brushite) after heat treatment in 1000 °C, at 20 wt% brushite ratio

brushite ratio from 20 to 25% due to the increase in β-TCP ratio, which is obtained during solid state reaction; there is slightly decrease in compressive strength at increase in the brushite ratio (25 to 40%) and this due to increase in the decomposition of brushite at high percentage ratio. HA/β-TCP mixture has higher mechanical properties than the single phases [15]. In this work, the average compressive strength of hydroxyapatite derived from bovine bones that sintering at the same previous conditions at porosity (29.3 vol %) and bulk density 2.225 gm/cm^3 was 55 MPa. The compressive strength of hydroxyapatite at particle size 160 μm and heating rate 10 °C/min and temperature 1000 °C with porosity 40% was (35 to 40 MPa) and this is close to compressive strength of native bone [16].

Figure 4 shows the effect of porosity on compressive strength at 20% brushite ratio; the increase in porosity attributable to addition of PEG at different ratio leads to decrease in all mechanical properties. This is because it represent a stress concentration on ceramic body, the compressive strength decrease in exponential manner with increase volume fraction of porosity and seem to be the same trend at all ratios of brushite addition Table 2.

SEM Results

The microstructure of the composite was observed using scanning electron microscope (SEM) instrument (VEGAII/ TESCAN XMV, Razi Center Laboratories, Tehran). Figure 5 shows the SEM result of HA/β-TCP scaffold at 10%, and 5% PEG addition, respectively, for surface and surface of fracture. It is clear that porous microstructure was obtained, and macroporous (d > 50 nm) with pore size ranging from few micrometers to nanometers, and the amount of porosity was increased as the PEG content increased due to the removal of polymeric content during firing and sintering process, the gaseous products generated from polymeric particle and binder combustion and decomposition process also from evaporation of moisture content contributed to produce pores. The fracture surface shows transgranular fracture with porous microstructure between grains and necks of connection of grains, which indicates that interconnection pores were produced due to sintering condition, PEG content, and the size of polymeric particles. The high sintering temperature was avoiding and sintering at short holding times to avoid the produce of dense microstructure in conventional pressureless sintering. This is because the high temperatures are associated with excessive grain growth and decomposition of HA. It has been reported that processing of HA at higher temperatures (exceeding 1250–1450 °C) result in exaggerated grain growth and decomposition, while large pores were produced in sintering bodies at 1050 °C and significant reduction in porosity at 1250 °C [17, 18].

Table 2 Experiments variables with it results

No. of experiment	Porosity (vol %)	Ratio of brushite (WT %)	Compressive strength(MPa)
1.	39.29254	20%	28.41803
2.	41.78916	20%	23.27124
3.	43.21552	20%	19.58491
4.	44.82809	20%	18.0936
5.	45.52036	20%	16.3
6.	41.2756	25%	53.32
7.	43.85346	25%	42.83574
8.	44.57394	25%	30.61803
9.	44.72708	25%	29.30465
10.	47.60899	25%	19.58491
11.	42.0503	30%	49.98675
12.	44.51532	30%	35.77768
13.	46.92993	30%	32.45
14.	47.588	30%	25.63872
15.	51.78152	30%	7.699725
16.	44.33717	35%	48.54006
17.	44.42091	35%	44.5468
18.	44.91411	35%	40.72336
19.	47.28097	35%	31.20337
20.	47.52203	35%	15.40669
21.	44.8227	40%	48.19474
22.	45.37852	40%	43.15328
23.	46.32837	40%	36.71919
24.	49.70557	40%	35.56117
25.	54.24297	40%	15.61087

Regression Equation Analysis

The prediction model for compressive strength is established by taking compressive strength as dependent variable and input variables (porosity, ratio of brushite) as independent variables. Therefore, the design of experiment has used based on 25 experiments for two factors and five levels. Table 2 using Minitab software, a regression relationship is established between them, given in Equation 8:

$$\sigma = 52.9 - 137\ P + 452\ rb - 632\ p*rb \quad (8)$$

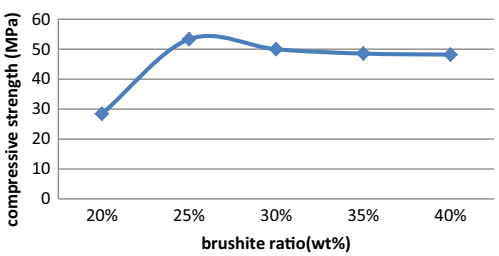

Fig. 3 Relation between compressive strength and brushite addition

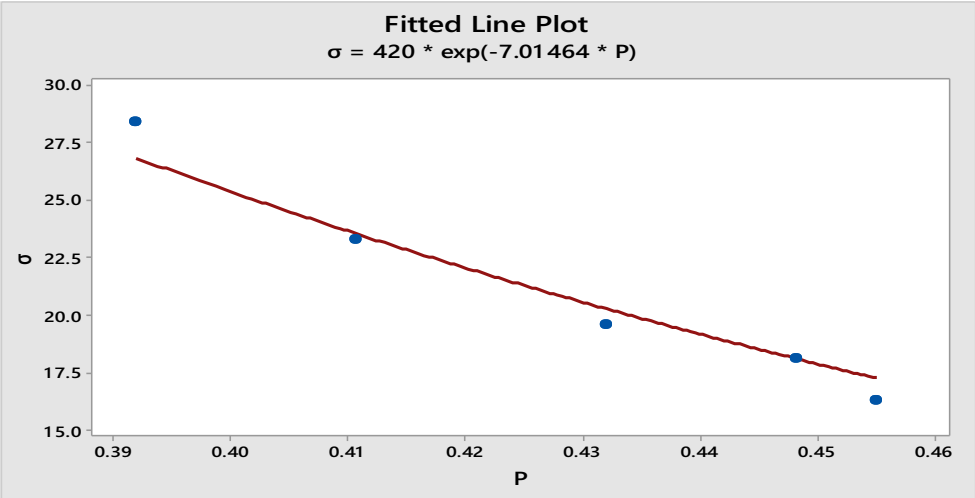

Fig. 4 Relation between porosity and compressive strength at 20wt% brushite

where (σ) is compressive strength, (rb) ratio of brushite, and (p) is total porosity.

This analysis in Table 3 *p*-value is close to zero, and the R2 value in statistics is also a key parameter; this is because it indicates model performance. For this reason, R2 = 73.89%, R (adj) = 70.16%, which is considereded to be a good fit between dependent and independent variables.

The Objective Function and Parameter of Genetic Algorithm

The first step of the GA is creation of initial population with individuals while the individuals represent a possible solution or chromosomes. The next step is evaluation of individuals by the use of fitness function. The population is then operated by

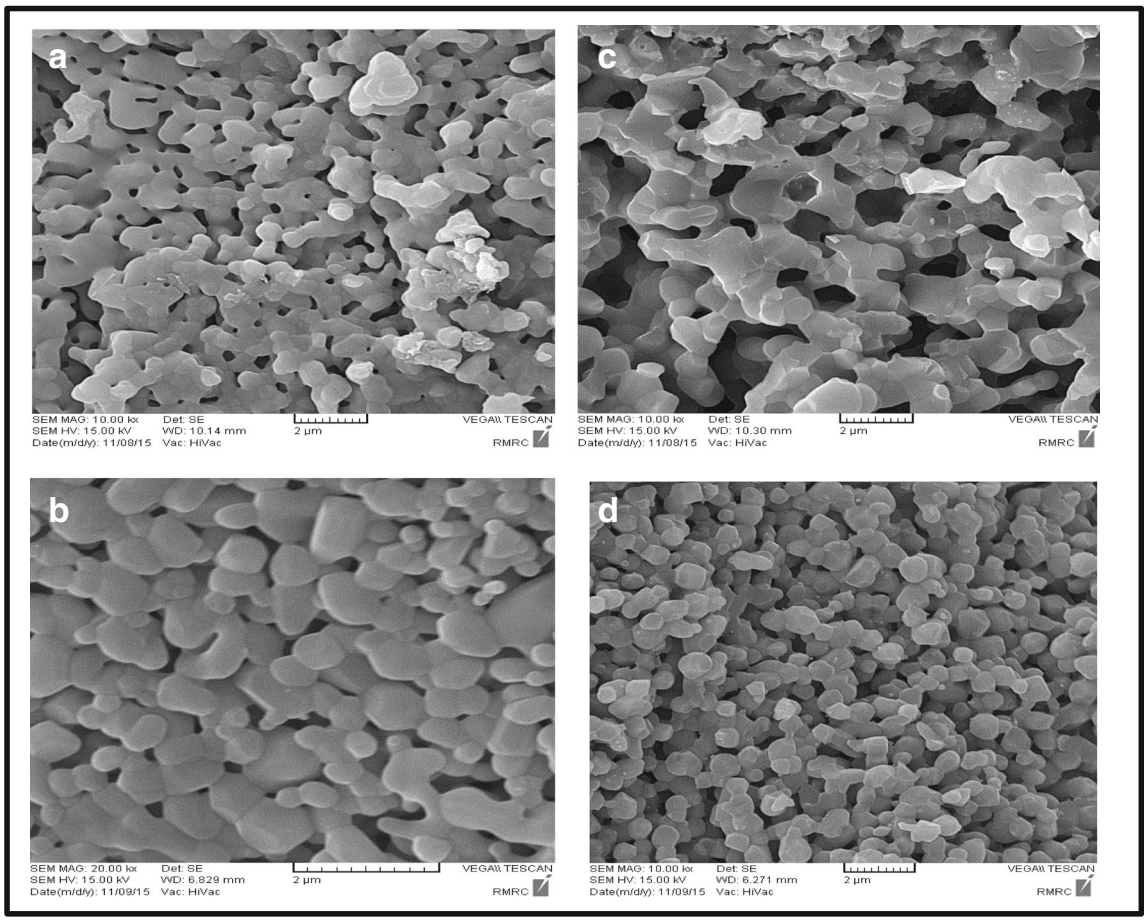

Fig. 5 SEM for HA/β-TCP scaffold for surface at **(a)**10%PEG; **(b)**5%PEG addition; and fracture surface at **(c)**10%PEG, **(d)**5%PEG

Table 3 Analysis of regression equation

Term	Effect	Coef	Se coef	*t*-value	*p*-value
Constant		27.94	1.74	16.03	0.000
Porosity	-49.10	–24.55	3.66	-6.70	0.000
Ratio	25.92	12.96	2.49	5.21	0.000
Ratio*porosity	-9.51	-4.76	4.56	-1.04	0.309

three GA operators (reproduction, crossover, and mutation) to produce new population of points [19].

The genetic algorithm option in the optimization tool of Matlap software was used to represent solution of the optimization problem. The parameters of GA were as follow:

- The objective function

The objective is maximizing the compressive strength with constrain porosity bounded by 40–50 and ratio bounded by 20–40. Equation (8) will represent the fitness function.

- Initial population and number of generation

The population consists of individuals or chromosomes that form the mating pool, the chromosome represented by parameters of problem, in this case porosity and ratio will represent the parameters or variables. The population type was bit string and their size was 20 individuals. As the number of generations increases, the average fitness value of the entire generation increases, and the individuals in the population get closer to the optimum point; the number of generation was 50.

- Selection crossover and mutation

The selection type was stochastic uniform, and the children created by combining the genes from a pair of parents selected on count of fitness. The two-point

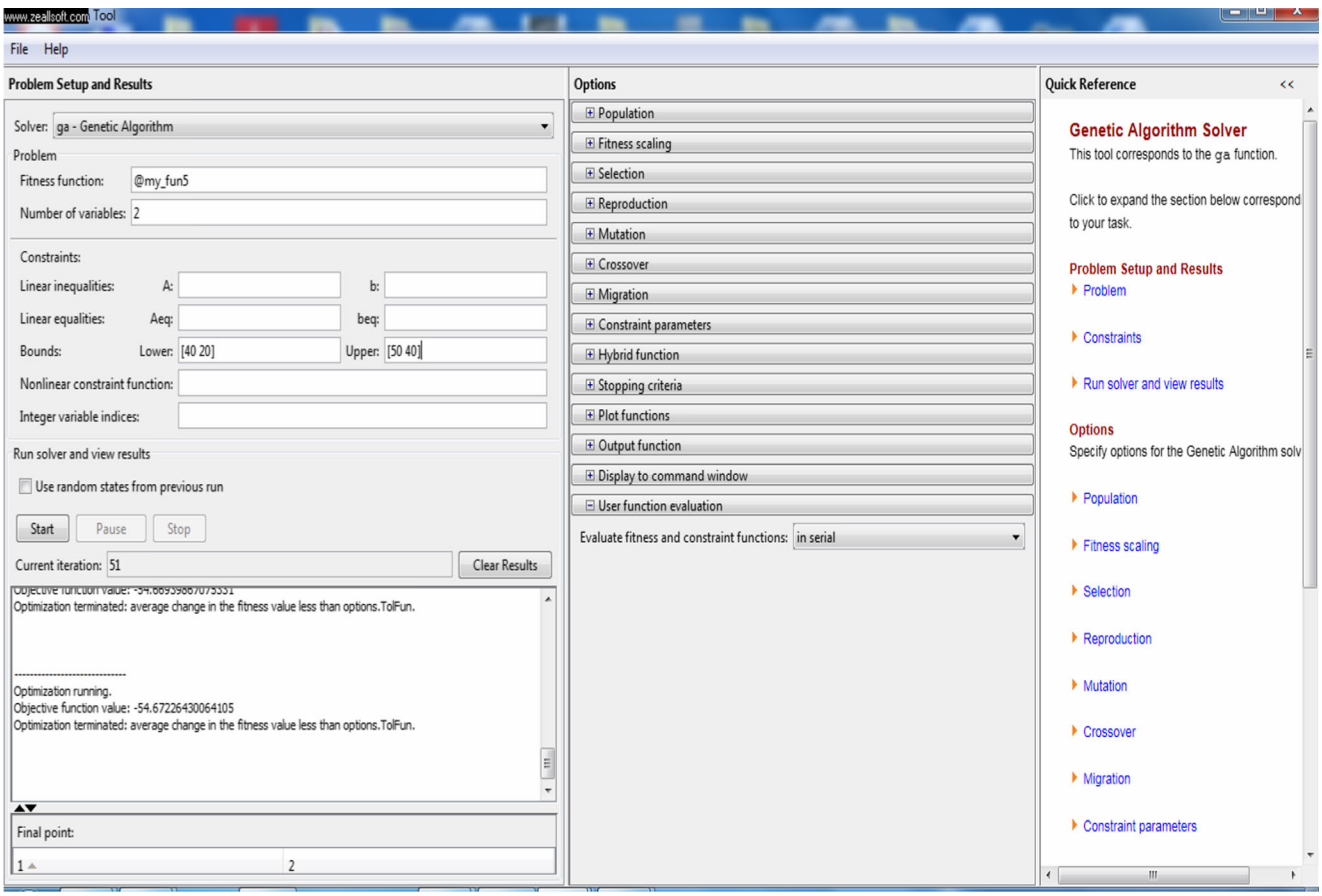

Fig. 6 The output solution by Matlap software

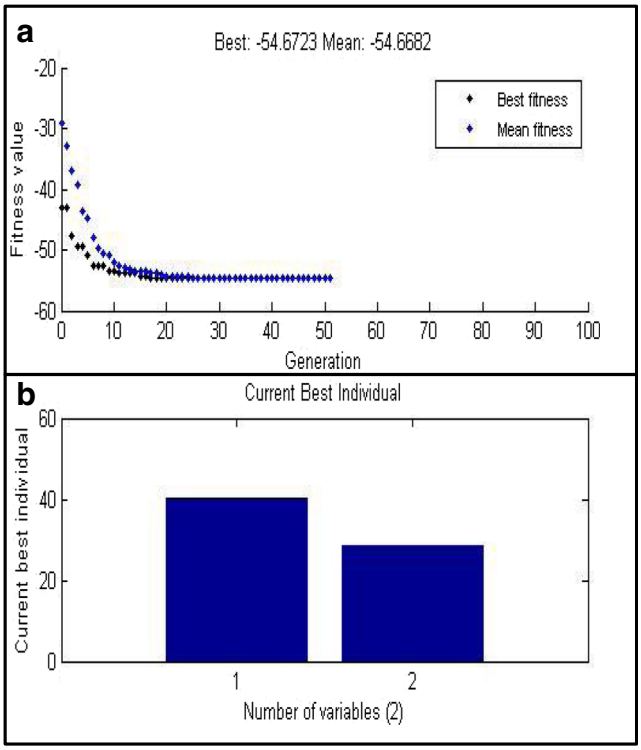

Fig. 7 **(a)** The fitness value versus the generation; **(b)**the best individual or chromosome

crossover is selected and crossover fraction was 80%, the children created by introducing random change or mutation in the gene of a single parent the mutation type was uniform with probability 20%.

The Output Solution

Figure 6 explains the objective value result in Matlap software, which was (54.67MPa) for compressive strength (Fig. 7a), which shows the fitness value versus generation, while **(**Fig. 7b**)** shows the best individuals or chromosome at this value of compressive strength. This is the result in approximate agreement with experimental value (53.3MPa), whereas the other factors of uncontrolled conditions, such as moisture, presence of agglomerate, and the morphology of porosity … etc. will lead to this difference.

Conclusions

HA/β-TCP scaffold was successfully prepared using sacrificial method, utilizing particle of polymer (PEG) as poregen. The porosity obtained was in the range 40–54% and it increased as the percentage ratio of polymer increased. The genetic algorithm optimization was used to optimize the problem parameters, which are involved porosity and the ratio of brushite phase. The best compressive strength using genetic algorithm was 54.67 MPa, in approximate agreement with experimental value 53.3 MPa; this may be due to uncontrolled condition such as moisture, presence of agglomerate, and the morphology of porosity and others factors.

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