

RESEARCH PAPER

Development of Porous Photopolymer Resin-SWCNT Produced by Digital Light Processing Technology Using for Bone Femur Application

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Abstract

Background: Although bone tissue has the unique characteristic of self-repair in fractures, bone grafting is needed in some situations. The synthetic substances that are used in such situations should bond to the porous bones, be biocompatible and biodegradable, and do not stimulate the immune responses. Biomaterial engineering is the science of finding and designing novel products. In principle, the most suitable biodegradable matrix should have adequate compressive strength of more than two megapascals. At this degradation rate, the matrix can eventually be replaced by the newly formed bone, and the osteoprogenitor cells migrate into the scaffold. This study aimed to evaluate the fabrication of a scaffold made of polymer-ceramic nanomaterials with controlled porosity resembling that of spongy bone tissue.

Methods: A compound of resin polymer, single-walled carbon nanotube (SWCNT) as reinforcement, and hydroxyapatite (HA) were dissolved using an ultrasonic and magnetic stirrer. A bio-nano-composite scaffold model was designed in the SolidWorks software and built using the digital light processing (DLP) method. Polymer-HA scaffolds with the solvent system were prepared with similar porosity to that of human bones.

Results: HA-polymer scaffolds had a random irregular microstructure with homogenizing porous architecture. The SWCNT improved the mechanical properties of the sample from 25 MPa to 36 MPa besides having a proper porosity value near 55%, which can enhance the transformation and absorption of protein in human bone.

Conclusion: The combined bio-nanocomposite had a suitable porous structure with acceptable strength that allowed it to be used as a bone substitute in orthopedic surgery.

Level of evidence: Therapeutic study

Keywords: 3-D Printing, Biocompatible materials, Carbon nanotubes, Hydroxyapatite, Tissue engineering

Introduction

One defects due to fractures, congenital anomalies, osteoporosis, and cancers are major challenges in orthopedic surgeries. With the increasing number of old population and the increased life expectancy, patients and orthopedic surgeons encounter an increased demand for artificial biomaterials to repair the large bone defects during the surgery (1-2). The biodegradable materials fabricated with engineering techniques are made of natural polymers and ceramics, which can be a bone substitute in bone defects and act as a suitable artificial tissue (3-4). Bone and tissue engineering aim to

investigate methods of fabricating new products, reduce side effects, and increase the efficacy of the fabricated products (4-6). In recent years, bioengineers are preparing and determining the best artificial composition with proper mechanical and biological characteristics for orthopedic surgeries in large bone defects (1-6). The polymeric-ceramic bionanocomposite is one of these new synthetic tissues with similar architecture to that of the real spongy bones (5-6). The application of different porous scaffolds with biocompatible and biodegradable materials that can accelerate the repairing process in

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the bone without any side effects to the adjacent tissues is still a matter of debate among bioengineers (3-4). Hydroxyapatite (HA) is the most important ingredient in bone tissue and a calcium phosphates (CaPs) biochemical agent with low mechanical performance including low fracture toughness and compressive strength. Varieties of research have been done on this material investigating the repairing process of bones using this substance (5-8). The HA powders are easy to transfer from implanted places; however, molding of HA is troublesome due to its hardness and brittleness. Therefore, scientists are trying to compensate and improve the poor mechanical properties of HA using new composites made of organic polymers (8-12).

Research has shown that single-walled carbon nanotube (SWCNT) can have an inductive effect on osteoblasts in the fracture site as a part of bioceramic composites other than its biodegradation capacity (13-16). These nanotubes have high thermal, electrical, and strength properties, which make them a promising candidate for increasing the mechanical properties of HA (12). Three-dimensional (3D) printers, space holders, freeze-drying, and hot and cold isostatic press method are different ways to make scaffold-shaped polymer nanocomposites (17-19). 3D printers can create complex porous specimens with high porosity to be used in spongy bone fractures (11). However, the feasibility and mechanical properties of 3D-printed porous-ceramic nanocomposites based on photopolymer resins for orthopedic applications have not been investigated up to date. This study investigated the mechanical strength of 3D-printed HA and the SWCNT bio-nanocomposite scaffold.

Materials and Methods

Fabrication of a hydroxyapatite-carbon nanotube bio-nanocomposite

Initially, 20 milliliters (ml) of ethanol was mixed with 20 ml of the dental photopolymer resin (Dental resin, DITAX Company with 360-410 nanometer (nm) wavelength and ultraviolet (UV) curing) to make a porous scaffold which was then placed on the magnetic stirrer for 45 minutes (min). Afterward, 0.5 gram (g) of nano-HA powder (Merck, Germany, 40-80 nm, 98% purity) was added to the composite solution and placed on a magnetic stirrer for 60 min with an ultrasonic bath at a temperature of 135°C. The combination of the composite solution blended with HA and photopolymer resin was prepared. Subsequently, 0 weight-percentage (wt%), 1 wt%, and 2 wt% of SWCNT was added to the HA-photopolymer solution and mixed with the magnetic stirrer at room temperature for 2 hours at 250 revolutions per minute (rpm) and a temperature of 135°C. Subsequently, 0.2 ml triethanolamine (TEA) was added to the mixed solution to create more surface tension, disperse the composite nanoparticles on the solution, and prevent agglomeration. The prepared nanocomposite containing 0 wt%, 1 wt%, and 2 wt% SWCNT solutions were inserted in the digital light processing (DLP) machine to print the porous architecture [Figure1]. The inter-structural distances in a scaffold model were set at 0.5 square millimeters (mm²). The models were designed with a cylindrical shape to

mimic the real human bone. The above design was done by SolidWorks software and saved as an STL file to be sent into the DLP printer software (creation Workshop) as shown in Figure 1. The drying time was 10 seconds per layer using UV light in which the height of each layer was set at 0.02 mm.

Characterization of the bio-nano composite scaffold

The phase analysis and chemical element analysis were performed on the three samples using x-ray diffraction (XRD) and X-ray fluorescence (XRF), respectively. The XRD device is mainly used to estimate the size of crystals in crystalline structures, along with its many other applications. The XRF is used to decompose superficial layers of the porous scaffold. This technique can be used to perform elemental analysis in qualitative and semi-quantitative formulas, especially in the case of mineral samples. The porous scaffold morphology was investigated using XL30 and Scanning Electron Microscope (SEM) tools in Amirkabir University of Technology, Tehran, Iran. Different magnification was applied to investigate the morphology of porous bone architecture. To prepare the nanocomposite scaffolds for cell culture assay, the HA combined with SWCNT and dental resin were sterilized and washed using 70% ethanol. Afterward, the samples dried in a clean room with ambient temperature. The fibroblast (HuGu) cells were cultured from human gums in a culture medium containing DMEM and cow serum within six polystyrene cavities. The adherent cells were washed away using PBS solution after seven days. The cells were grown and cultured under controlled conditions (95% air, 5% carbon dioxide) at 37°C and the culture medium was changed once every two days. In the next step, 10,000 cells were cultured in the plate. The porous scaffold made in DMEM solution containing 15% FBS was incubated for seven days. A positive response to cellular toxicity indicated a

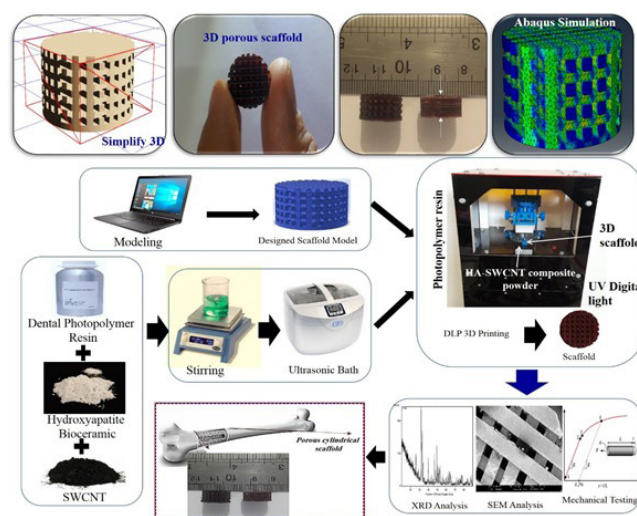


Figure 1. Schematic preparation of porous scaffold made of SWCNT and HA in the dental photopolymer resin after two hours of ultrasonic bath and fabricated by digital light processing -3D printing.

lack of biocompatibility, while a negative response (non-toxicity) does not necessarily represent a biocompatible property.

Mechanical investigation of the 3D-printed scaffold

The compressive strength (CS) test was used for the porous HA-SWCNT composite samples using SANTAM-STM50 with a load cell of 10-20 kilonewtons (KN) following ASTM-D5024-95a standard. The dimensions of each specimen were 10×10×10 cubic millimeters (mm³) with a speed of 0.5 meters per min. The elastic modulus was calculated from the slope of the stress-strain curve, which was considered as the amount of compressive strength under the compression test. Samples were tested three times to ensure the accuracy and precision of the obtained data. All the fabrications were done based on try and error and five times repetition. The porosity of the scaffolds was measured following Archimedes' principle using Image-J software. Porosity measurements provide information on the size and distribution of pores, permeability, and the presence of structural imperfections in spherical ceramic structures. In this method, due to the hydrophobic nature of the polymer, ethanol was used to penetrate easily into fine porosities. The amount and the volume (V_1) of ethanol in the cylinder were measured respectively. The sample was then put in ethanol for five minutes to be completely saturated. The volume measured after saturation is shown by V_2 . The difference between the two volumes ($V_2 - V_1$) represents the size of the scaffold. The ethanol-impregnated scaffold was removed from the cylinder and the residual volume is shown by V_3 . The amount of $V_1 - V_3$ is known as the volume of ethanol absorbed by the scaffold. Therefore, the final volume of the scaffold is calculated based on Equation 1 below:

$$V = [(V_2 - V_1) + (V_1 - V_3)] / (V_2 - V_3) \quad (1)$$

Micromechanical model of the porous bony scaffold

According to the mechanical results, several conventional models were developed on the porous scaffolds to repair damaged bone with new hard composite tissue made of the DLP technique. However, the acquisition of mechanical properties of such porous scaffolds using laboratory methods is very time-consuming and costly. Many researchers have focused their studies on mathematical methods, such as elastic modulus and porosity ratio to predict the mechanical properties and most of the previous models were based on the finite element method (FEM). In this paper, various micromechanical methods have been introduced to obtain the effect of porosity on the elastic modulus of bone scaffolds. Single-scale ceramic scaffold modeling and multi-scale composite scaffold modeling were performed using Abaqus software. The mechanical properties of HA in different porosities were presented in the current study due to the widespread use of HA in bone scaffolds. The conventional micromechanical model was used to evaluate the elastic modulus of porous (E_p) and elastic modulus of solid (E_s) versus porosity of the bone using Dewey models, as indicated

by Equation 2 and 3.

$$\frac{E_p}{E_s} = 1 - \xi\phi \quad (2)$$

$$\xi = \frac{(1 - \nu_s)(27 + 15\nu_s)}{2(7 - 5\nu_s)} \quad (3)$$

Results

Figure 2 demonstrates the XRD spectra of HA, SWCNT, and the composition of HA-SWCNT from 20° to 80°. Based on the observations, the obtained porous scaffold was made of a single-phase HA, and no additional sustained phase was detected. The main peaks of XRD for HA powder on plane "211", "112", "300", and "202" were similar to the natural bone, indicating its medical usefulness. The agglomeration of SWCNT in the photopolymer resin matrix leads to a different mechanical performance proven by micromechanical modeling. The XRD pattern showed that the HA powder had a purity of 96% and its mineral impurities included Na₂O (1 wt%) and MgO (1 wt%). Some researchers have evaluated the mechanical features of a similar composite from 20 to 60 MPa (19-33). The XRD pattern showed that the intensity of composite powder was between the intensity of the single-crystal HA and the SWCNT powders. The HA-SWCNT was synthesized at 30-40° with an intensity of 300-400 rpm.

The SEM images of the fabricated scaffolds are shown in Figure-3 (a-c). The status of a porous scaffold plays an important role in cell functions. Based on the obtained results, the porosities of these scaffolds are in the range of 100-300 micrometers that could improve cell proliferation and extracellular matrix (ECM) by stem cells of bone marrow (BMSCs). Figure 3 (d) indicates the properties of cultured cells in the samples that contained 0 wt%, 1 wt%, and 2 wt% SWCNT in the bio-resin-HA matrix after seven days. The obtained results suggested

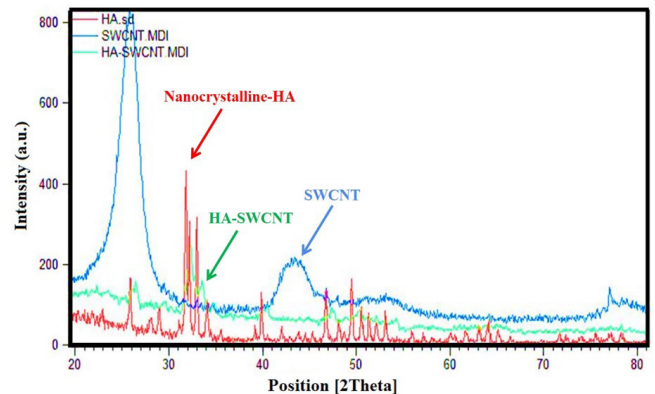


Figure 2. X-ray differentiation pattern comparison of crystalline nanocrystalline HA, single-walled carbon nanotube, and HA-SWCNT composite.

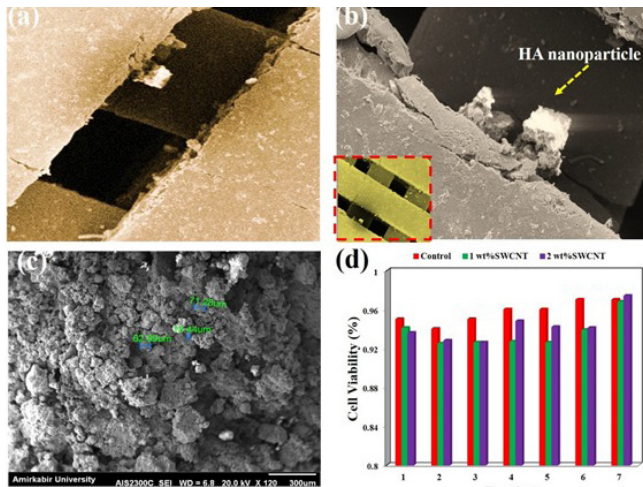


Figure 3a- d. SEM images of (a) porous scaffold fabricated using digital light processing technique, (b) soaked sample in the PBS solution, (c) the HA nanopowders to determine the size and distribution, (d) cell culture property of the samples containing 0 wt%, 1 wt%, and 2 wt% SWCNT after seven days incubated in osteoblast cell.

that the 1wt% SWCNT sample had good biocompatible behavior compared to other samples.

As indicated by Figure 4, the CS evaluation revealed that adding 1 wt% SWCNT increased the CS by more than 30%. The CS diagram increased slowly by nearly 36 MPa after adding 2 wt% SWCNT. The CS analysis indicated that the elastic modulus of the SWCNT is higher than that of HA bioceramic which affects the mechanical properties of the matrix. The obtained CS was measured at 25, 33, and 36 MPa for a sample

containing 0, 1, and 2 wt% SWCNT, respectively. In this study, the porosity did not change extensively regarding the accuracy of the DLP technique, which was near 100-150 micron for the cell growth and improvement of growth factors. The porosity of the sample increased from 55 to 61% after SWCNT was increased up to 2 wt%, as shown in Figure 4(b).

Figure 5 (a-b) compares the rate of elastic modulus of the solid form to the elastic modulus of the porous form (E_s/E_p) with the porosity value for bio-nanocomposite containing various amounts of SWCNT. The micromechanical model performed well and the scaffold was tailored to the size of the lesion using the rapid prototyping technique [Figure 5]. Considering the elastic modulus, the obtained porosity rate indicated that a good porous bone was produced and would be bonded with surrounding tissues when its fracture strength reaches 85% of the normal bone. The microhardness might be influenced by the amount of porosity of the pore size and strut size. The results of the XRF analysis on the sample of HA powder are presented in Table 1. These XRF results confirmed the presence of the HA with the SWCNT phase with high purity. It was found that calcium and phosphorus are the main elements of the HA powder. Moreover, impurities in the HA structure included sodium and magnesium ions.

Discussions

HA composites can be employed to repair bone defects in bone tissue engineering sciences. The microstructure of this ceramic has weak mechanical properties and low chemical stability that needs to be reinforced and strengthened using such compounds as carbon nanotube, titanium, and zirconium nanoparticles (15-18). It is not possible to make a nanocomposite

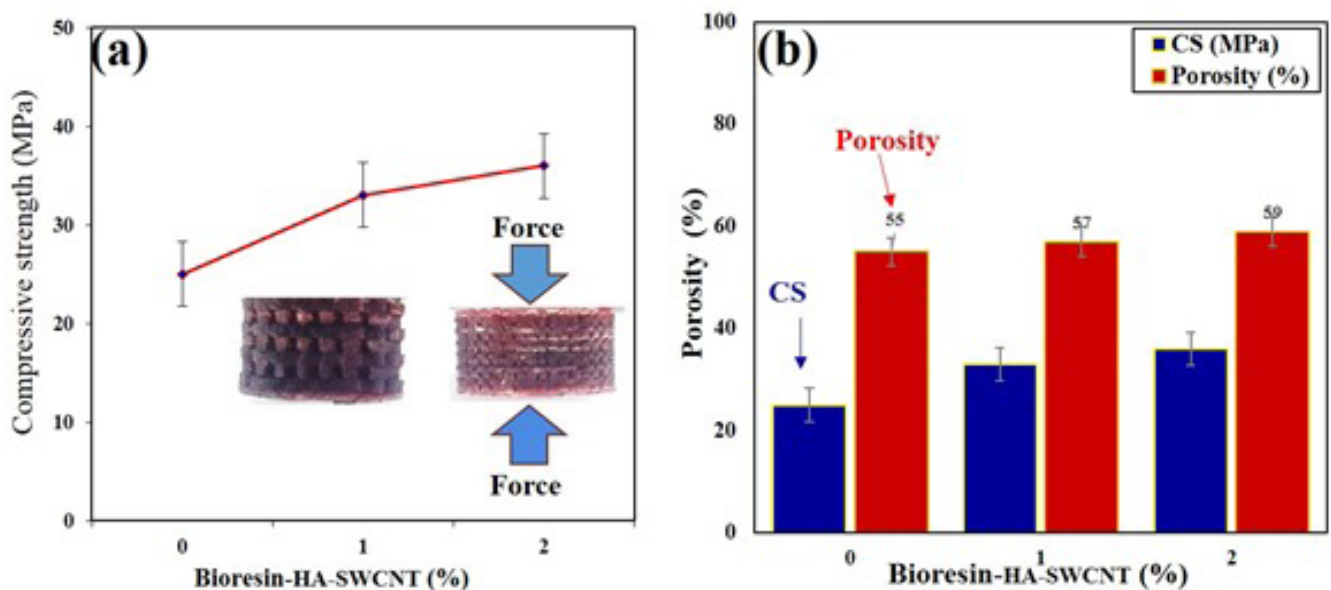


Figure 4a,b. Analysis of (a) Compressive strength, and (b) compressive strength vs. porosity value of the porous scaffold designed using digital light processing technique made of HA and SWCNT soaked in the photopolymer resin.

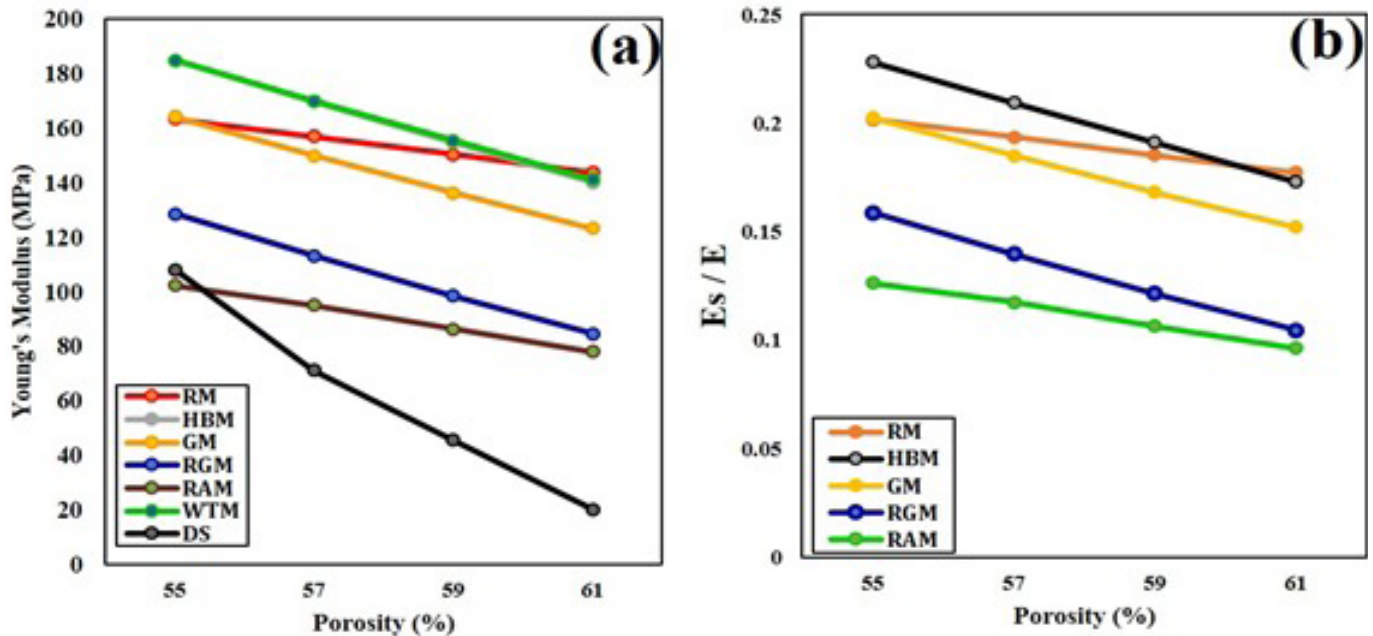


Figure 5a, b. Micromechanical model used to predict the compressive strength of various porous architecture (a) elastic modulus vs. porosity, (b) E_s/E_p vs. porosity value for bio-nanocomposite with various amount of SWCNT.

containing HA and SWCNT without dispersant since the base and reinforcement nanoparticles deposit rapidly to overcome the bioceramic weight. The use of dispersant prevents this event and leads to proper homogeneity. The addition of different quantities of SWCNT nanoparticles can enhance the basic structure of artificial bone designed by the DLP technique. The addition of SWCNT increases the CS of the specimen by more than two times. Generally, the porosity of the samples in this method was between 55% and 61%,

Table 1. Elemental or spectral analysis of X-ray Fluorescence (XRF) spectroscopy of HA nanoparticles

Elements	(%w/w)
CaO	55.2
P ₂ O ₅	37.5
Na ₂ O	1.7
SO ₃	1.6
MgO	1.4
SiO ₂	1.2
Cl	0.89
H ₂ O	0.83
Al ₂ O ₃	0.65
SrO	0.60
K ₂ O	0.55
C	0.42-0.8
CuO	0.40

which was notable. The addition of 1 to 2 wt% SWCNT results in optimal porosity and compressive strength. The results of a study conducted by Saber-Samandari et al. showed that bone scaffold had significant mechanical and biological properties (17). They synthesized a porous bone tissue composite with intrusive phases of collagen (polymer) and HA (biochemical). The scaffold fabricated by the DLP technique presented proper mechanical, biocompatibility, and bioactivity properties and showed remodeling capabilities. The HA nanoparticles improved the properties of the scaffold in several ways due to high specific surface area and apatite formation (29-33). In terms of tissue engineering, the reinforced nanocomposite with these bioactive nanoparticles had an increased mechanical performance. In addition, reduction of the particle size to the nanoscale can increase the structural property of these scaffolds given the known biological properties of HA in terms of bone conductivity and bone growth. The results of micromechanical model simulation showed that the designed models had the same average load-bearing in terms of power distribution, which can transfer function uniformly (31-32). However, a porous structure scaffold exhibited greater endurance against the same load in terms of the stresses entailed in the scaffold model, which is a significant positive factor. There are two main reasons for the implementation of this scaffold in the body. One is the higher load tolerance that makes it more efficient and safe. Therefore, the probability of failure is less even with the application of excessive force. Moreover, due to higher porosity and spacing between the scaffold columns, collagen and HA are more scalable in this model than other

micromechanical models. Therefore, it is expected that this model can accelerate the bone repair process. The attached cells derived from MSC can be differentiated into the osteoblast embryonic stem cells (OESCs).

It should be noted that the internal evaluation of these scaffolds is ongoing in an animal study. The CS diagram indicates that the strength of the polymer composite increases with an increased portion of SWCNT. With the increase of SWCNT, elastic modulus (E) and ultimate tensile strength (UTS) are incremented as a result. In other words, the composites with a higher concentration of SWCNT are more rigid and become less deformed. The fracture models indicate more ductile performance through the stress and strain curves. The CS of ceramic scaffolds depends on the size and geometric shape of the porosity. In the same line, Karbasi et al. added different amounts of CNT to poly-3-hydroxybutyrate (P3HB) (19). They obtained proper structural properties for soft and hard tissue applications. Vatankhah et al. produced novel composite scaffolds based on Tecophilic (TP) that proliferated the rate of the smooth muscle cells (SMCs) (20). They accomplished using this tissue for vascular grafts with adequate cell growth. In another study, researchers enhanced the weak mechanical properties of architecture using natural urinary bladder ECM with polycaprolactone (PCL) polymer. The manufacture of geometrically complex nanocomposites containing magnetic nanoparticles (MNPs), which can be used as thermal therapy or hyperthermia against cancerous cells, is one of the prominent features of the DLP method compared to other techniques for the preparation of scaffolds (24-31). This study aimed to demonstrate the capacity of a 3D printing technique, DLP, in the production of HA scaffolds (28-32).

Given the capabilities of the DLP-3D printing technique in the manufacturing of complex bone components, materials such as photopolymer resins, HA, and SWCNT can be used to create models of bone scaffolds. These bony scaffolds with sufficient porosity can accelerate

the bone repairing process. The fabricated scaffold can be adopted as a suitable porous structure in orthopedic surgeries. The scaffold presented in this study can repair the small bone defects following orthopedic surgery. In addition, the obtained results indicated that the sample containing 1 wt% SWCNT presents proper mechanical and chemical features compared to other samples.

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