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# Mechanical and biological performance of printed alginate/methylcellulose/halloysite nanotube/polyvinylidene fluoride bio-scaffolds

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ABSTRACT

Use of artificial cartilage due to its poor regenerative characteristics is a challenging issue in the field of tissue engineering. In this regard, three-dimensional printing (3D) technique because of its perfect structural control is one of the best methods for producing biological scaffolds. Proper biomaterials for cartilage repairs with good mechanical and biological properties and the high ability for 3D printing are limited. In this paper, a novel biomaterial consisting of Alginate (AL), Methylcellulose (MC), Halloysite Nanotube (HNT), and Polyvinylidene Fluoride (PVDF) was printed and characterized for cartilage scaffold applications. Calcium chloride ($CaCl_2$) was used as a crosslinker for biomaterial after printing. Scanning Electron Microscopy (SEM), Energy-Dispersive X-Ray Spectroscopy (EDX), X-Ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FT-IR), Differential Scanning Calorimetry (DSC), tensile and compressive tests, chondrocytes seeding, cells staining, and MTT assay were carried out in the present work. The results show that in constant concentrations of AL, MC, and PVDF (40 mg/ml AL, 30 mg/ml MC, and 1% PVDF) when concentration of HNT increased from 20 mg/ml (S2) to 40 mg/ml (S14) tensile strength increased from 164 up to 381 kPa and compressive stress increased from 426 up to 648 kPa. According to spectroscopy and calorimetry results, Biomaterial shows an amorphous structure with good miscibility and a high percentage of water in its structure. PVDF reduces mechanical properties by 7% while increases cell viability by 8.75%. Histological studies and MTT assay results showed a high improvement in the percentage of living cells at the first 4 days of cell cultivation.

## 1. Introduction

The most important goal of tissue engineering is to produce appropriate bio-scaffolds with good mechanical behavior (such as living tissues) and biocompatible properties [1]. Among all available methods to build biological scaffolds, three-dimensional (3D) printing technique provides good structural control and interconnectivity, making it one of the bests for producing biological scaffolds [2]. In addition, early studies show that architecture and composition of these scaffolds can strongly influence cell adhesion and proliferation and cause different biological actions [3, 4].

Cartilage is a poroelastic material considering the strong relationship between time and its deformation [5]. The water content of cartilage is about 70–85%. Since biomaterials with large water uptake ability are needed for printing of cartilage bio-scaffolds [6]. Cartilage is an avascular tissue with restricted self-regenerative properties and hard to regeneration by normal biomaterials [7, 8]. According to the previous studies, chondrocytes existing in natural cartilage are one of the best cells that can be used for cartilage repairs [9–11].

Biomaterials used for scaffolding can be divided into two groups of native and synthetic materials [12]. Native materials like fibrin, alginate, cellulose, and collagen have good biological properties such as easy biodegradation and high adhesion with chondrocytes; however, their poor mechanical properties hinder their effective utilization. Besides, synthetic biomaterials such as polycaprolactone (PCL), poly (lactic-*co*-glycolic acid) (PLGA), and the polymer of lactic acid (PLA) has effective mechanical characteristics in scaffolding but poor biological performance compared to native materials [13, 14]. Composite materials have an advantage over single materials that could meet the biological and mechanical properties needed for bio-scaffolds [15, 16]. Wang et al. [17] showed the high ability of alginate in cartilage tissue engineering but reported that pure alginate has poor mechanical properties. Min Seong Kim et al. [18] studied PCL/Alginate scaffolds and reported better mechanical properties in scaffolds with composite biomaterials in comparison with pure alginate. High improvements in shape and size stability in the printing of the bio-ink composed of

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nanofibrillated cellulose and alginate (NFC-A) was reported by Hector Martinez Avila et al. [19]. Alginate/methylcellulose scaffolds showed better elasticity and stability compared with pure alginate as well as improved micro porosities made by the release of MC [20]. Liu M et al. [21] studied alginate/halloysite nanotube composite scaffolds and reported that HNTs improve the thermal stability in this composite and also increase the stability of alginate scaffolds. Afshar et al. [22] reported remarkable increases in compressive properties of chitosan/alginate/Halloysite scaffolds in comparison with chitosan/alginate scaffolds.

Alginate (AL) with the formula '$(C_6H_8O_6)_n$' is a polysaccharide taken extracted brown seaweed. Permanent cultures in alginate scaffolds have been attained with chondrocytes that have the proper ability in bone healing and cartilage repairs [23, 24]. AL is a biocompatible material that can be adjusted to a wide range of viscosity for better print conditions [25]. The most common method to prepare hydrogels from an alginate solution is to combine the solution with ionic cross-linking agents, such as divalent cations (i.e., $Ca^{2+}$) [26, 27]. Calcium chloride ($CaCl_2$) is one of the most frequently used agents to ionically cross-link alginate and producing egg boxes [28, 29]. AL transfers to a stable hydrogel by making the ionic interaction between carboxyl groups of AL and $Ca^{2+}$ [30]. Methylcellulose (MC) is a favorable biomaterial in tissue engineering that improves mechanical properties of scaffolds such as tensile strength. MC can be used for printing scaffolds using resilient and smooth elastic tissues such as cartilage. Biocompatible properties and high cell viability make MC a proper biomaterial in tissue engineering [31, 32]. Halloysite Nanotube (HNT) with the formula '$Al_2Si_2O_5(OH)_4{\cdot}H_2O$' is an aluminosilicate clay that contains aluminum, silicon, and hydrogen. HNT is a safe, biocompatible, and environmentally friendly Nano clay [33] that can increase the wetting abilities of composite scaffolds and improve the adhesion of cells [34]. HNT reduces strains and increases mechanical properties of biomaterials [35]. The existence of polyvinylidene fluoride (PVDF) with the formula '$-(C_2H_2F_2)_n-$' causes an electrical charge with positive effects on cell growth and cell adhesion in scaffolds [36–38]. Electrical impulse has a good effect on cell attachments to scaffolds. Several studies show that electrical impulses can attract cells and improve adhesion. Actually, electrical impulses sourced from PVDF, by absorbing cells with opposite polarity around them, cause more adhesion to the body of the scaffolds [39].

According to recent studies, it is seen that there are not abundant of biomaterials meeting both mechanical and biological performances. In this study, we describe the printing of cartilage scaffolds with a novel biomaterial that contains a true combination of AL/MC/HNT/PVDF. Combination of alginate as a native biomaterial with MC improves the printability and elasticity of scaffolds. Moreover, the addition of HNTs to structure for enhances the compressive properties of the biomaterial. Finally, the addition of PVDF in biomaterial to study the influence of PVDF on the cell attachments makes a novel biomaterial that can be used in tissue engineering and scaffolding. Calcium chloride ($CaCl_2$) was used as a crosslinker for biomaterial after printing. The present study was conducted to enhance the efficiency of cell seeding in hydrogel-based printed bio-scaffolds both from mechanical and biological aspects.

## 2. Materials and methods

Alginic acid sodium salt (MW ~150–250 kDa), methylcellulose (MW ~90 kDa), polyvinylidene fluoride (MW ~530 kDa), trypsin, calcium chloride ($CaCl_2$), and 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) were purchased from Sigma Aldrich (Germany). Halloysite nanotube (MW ~294 kDa) was purchased from Naturalnano (Rochester, NY). Human chondrocytes cell lines were purchased from Pasteur Institute (Tehran-Iran). Lipophilic vital dye Dil was purchased from Biottium (USA). Glutaraldehyde was purchased from Proscitech (Germany). Other solutions and materials such as phosphate buffered saline (PBS), HEPES buffered saline (HBS), dimethylformamide (DMF), formaldehyde, paraformaldehyde (PFA), alcian blue, glacial acetic acid, and deionized water were purchased from Sigma Aldrich (Germany). Hematoxylin, eosin and toluidine blue were obtained from Merck (Germany). Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), and dimethyl sulfoxide (DMSO) were acquired from Gibco (UK).

### 2.1. Preparation of biomaterial

AL was stirred for 12 h with concentrations of 30, 40, and 50 mg/ml in PBS. Then, MC powder was obtained in 30 mg/ml concentration of MC/PBS solution and stirred for 24 h. The obtained MC solution was added to the prepared AL solutions under stirring for 12 h. Afterward, HNT solutions were prepared in concentrations of 20, 30, 40, and 50 mg/ml in deionized water. AL/MC and HNT solutions prepared with a ratio of 1:1 (AL/MC:HNT) were mixed and stirred for 12 h. Then, the mixtures were incubated for swelling of HNT and MC for 4 h. DMF was used as a solvent for PVDF solution in a concentration of 20:80 (PVDF: DMF) (w/v); next, it was mixed with the prepared mixture (AL/MC/HNT) at the ratio of 1:100 and stirred for 8 h to obtain a homogeneous mixture. Afterward, biomaterial was sterilized under ultraviolet light for 80 s. All of the experiments were performed at room temperature (37 °C). The prepared materials were stored at a temperature of 2–8 °C. Fig. 1 and Table 1 show the biomaterial preparation procedure and prepared compositions.

### 2.2. Three dimensional printing of bio-scaffolds

The bio-printer fabricated by the authors was used for scaffold printing. The printer has a clean printing box and a 10-μm accuracy (Fig. 2). Layers were oriented at 0°/90° with respect to each other. The thickness of each layer was 0.3 mm and line spacing was 300 μm. A 0.3 mm flat needle was used for printing. Several printing speeds

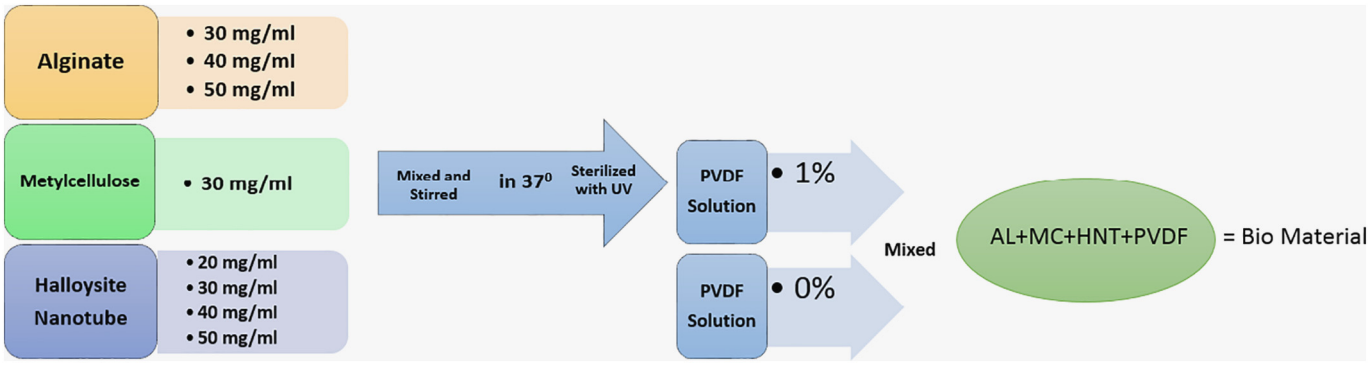


**Fig. 1.** Schematic steps of biomaterial preparation.