



Biocompatibility and biomechanical characteristics of loofah based scaffolds combined with hydroxyapatite, cellulose, poly-L-lactic acid with chondrocyte-like cells

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ABSTRACT

The current study reports the biocompatibility and biomechanical characteristics of loofah-based scaffolds combined with hydroxyapatite (HA), cellulose, poly-L-lactic acid (PLLA) with chondrocytes-like cells. Scanning electron microscope (SEM) micrographs of the scaffolds showed that the addition of PLLA usually resulted in an increase in cell's attachment on scaffolds. Mechanical and elemental analyzes were assessed using tensile test and Energy Dispersive X-ray spectrometry (EDS), respectively. In summary, we showed that the loofah + PLLA + HA scaffolds perform significantly better than other loofah-based scaffolds employed in terms of increasing a diversity of mechanical properties including tensile strength and Young's modulus. Based on the analysis of the differential scanning calorimetry (DSC) thermograms and EDS spectrums that give an idea about the calcium phosphate (CaP) ratios, the improvement in the mechanical properties could principally be recognized to the strong interaction formed between loofah, PLLA and HA. The viability of chondrocytes on loofah-based scaffolds was analyzed by XTT tests. However, none of the scaffolds have proved to be toxic in metabolic activity. The histological evaluation obtained by hematoxylin and eosin (H&E), Masson trichrome, toluidine blue and immunohistochemistry methods showed that cells in all scaffolds produced extracellular matrix that defined proteoglycan and type I-II collagens. The results of this study suggest that the loofah-based scaffold with desirable properties can be considered as an ideal candidate for cartilage tissue engineering applications.

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1. Introduction

The adult cartilage with limited healing potential cannot generally regenerate after injury. The difficulties in repairing damaged cartilage, therefore, forced the scientists to develop tissue engineering methods for the regeneration of injured tissue. One approach is to use biomaterials as scaffold reservoirs for cells and for mediators to stimulate cells to regenerate the tissue. Thus, biomaterials should not only be biocompatible but also be able to release the required mediators at different points in time to support tissue regeneration [1–3]. Cartilage engineering for aesthetic or reconstructive purposes relies on the same foundation of scaffold-cell interactions found in all tissue engineering applications [4].

Adhesion of cells to the surface of a biomaterial is one of the major factors defining its biocompatibility. Tissue engineering experimental methods investigating cell adhesion mostly rely on studying the morphology of cells and biomechanical characteristics of scaffolds in the absence or presence of cells [5].

PLLA is a nontoxic, biodegradable material which is widely used as a scaffold material in tissue engineering. Generally, most of the poly-L-lactic acid (PLLA) scaffolds have dense and smooth surface morphology [6]. As a scaffold material, PLLA also has several obvious weaknesses such as quick biodegradation, acidic degradation products [6]. One of the surface characteristics of synthetic polymers is hydrophobicity, influencing their biocompatibility unfavorably during the initial stage of contact with the biological environment [5]. Tavakol et al. reported HA to be an incredible nanocomposite, due to its superior biocompatibility, bioactivity [7]. A biodegradable polymer has the advantage that it could be easily removed from the body after the desired function by degradation without the need for surgical removal which increases patient

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comfort with reduction of cost for treatment [8,9]. On the other hand, inorganic scaffold composites are preferred because they combine stiffness and flexibility. Unlike polymers, HA performs multiple functions, such as releasing bioactive molecules for a prolonged period and aiding space filling for reconstruction and or regeneration of living tissues in the local area [6,10]. n-HA/polymer composite scaffolds such as n-HA/collagen, n-HA/gelatine, n-HA/polyamide, n-HA/poly-L-lactic acid and n-HA/poly-lactide-co-glycolide have been reported in the process of designing new scaffolds for cartilage tissue engineering [11–18].

Cellulose with linear homopolymer of glucose ($C_6H_{10}O_5$) with “n” number of glucose molecules ranging from 500 to 5000, is the most prevailing polymeric material in nature. It is degradable by enzymes [19,20] and its solubility in water depends on its chain length of “n” [19,21]. Cellulose fibers have advantages such as being abundantly available, low weight, biodegradable, cheap, renewable, low abrasive nature. They also exhibit good mechanical properties. They are waste biomass and are used as potential reinforcing materials [22–24]. On the other hand, cellulose fibers have disadvantages such as moisture absorption, quality variations, low thermal stability, and poor compatibility with the hydrophobic polymer matrices [22,25,26]. The chemical modification of cellulose has been investigated by many researchers and modified celluloses are used for many purposes [27,28].

Cellulose fibers have been in use by several research groups as reinforcing material to provide various types of composites. These composites further become less interesting to be used as they lack good interfacial adhesion and have low melting point and water sensitivity. However, it is possible to modify the fiber surface, to cease the moisture absorption process and increase the surface roughness via pre-treatment of the cellulose fibers using chemical functionalization [22,29].

The *Luffa cylindrica* (LC), best known as loofah or sponge gourd, has a ligneous fibrous netting system in which the fibrous cords are disposed of in a multidirectional array, forming a natural mat. The loofah fiber is one of the natural fibers, and it consists of cellulose molecules. The main chemical components of the loofah fibers are cellulose/hemicellulose and lignin. Recently, the loofah sponge has been shown to be a very efficient carrier for bio-immobilization [27–29]. Bal et al. reported that using the random distribution of fibrous strands in this natural netting system material in composite applications may be very advantageous [30,32,33].

The objective of the present study is to investigate the natural and novel loofah based scaffolds with different structures and their effects on the morphology and proliferation capacities of chondrocytes in vitro.

2. Materials and methods

2.1. Scaffold preparation and characterization

2.1.1. Preparation of simulated body fluid (SBF)

SBF contents were listed in Table 1 [34,35]. List of chemicals used in the preparation of SBF was as follows: (1) $CaCl_2 \cdot 2H_2O$, (2) $MgCl_2 \cdot 6H_2O$, (3) KCl, (4) NaCl, (5) $Na_2HPO_4 \cdot 2H_2O$, (6) Na_2SO_4 , (7) $NaHCO_3$, (8)

lactate and (9) lactic acid. In $SBF \times 2.5$; Ca^{+2} and PO_4^{-3} concentrations were 2.5 times higher compared to the concentrations in Table 1. To prepare SBF; chemicals numbered 3–8 were added in a container with 900 ml of distilled H_2O and then the pH was adjusted to 8.05 while the solution was stirred on a heated magnetic mixer. Seven millilitres of lactic acid (1 M) was quickly added, the temperature was increased to 37 °C quickly and pH was adjusted to 6.95. Salts with numbers 1 and 2 were dissolved in 10 ml distilled H_2O and added to the first solution quickly and pH was adjusted to 7.37. The final volume was completed to 1 l.

2.1.2. Loofah + HA

HA coating procedure on loofah is a new procedure and has been developed by Yuksel et al. [34,35]. We dissolved compounds numbered 3–8 (see in the preparation of SBF) in 800 ml pure water (pH: 8.1–8.2), heated the solution at 37 °C and then added 5 ml of lactic acid (1 M). $CaCl_2 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ salts were dissolved in 100 ml and added on to the previous solution. pH of the solution was adjusted to pH = 7 by adding lactic acid and total volume was completed with water till 1 l. Natural loofah material was purchased commercially. The loofah was soaked in this $SBF \times 2.5$ solutions in a beaker till it is fully coated with liquid and kept at room temperature for 9 days. The loofah coated with HA was then separated from the liquid, washed with water and dried at 50 °C.

2.1.3. Loofah + PLLA + HA

Loofah was soaked to swell and washed with water, then dried with NaOH (2 M). It was further soaked in THF (tetra hydra furan) and kept overnight in solution as loofah does not wet in chloroform. Finally, the loofah was dipped in 4% (a/v) PLLA (Fluka, 81273) solution in chloroform, dried and coated with $HA \times 2.5$ as previously described.

2.1.4. Loofah + cellulose + PLLA + HA

Loofah was first mixed with cellulose (Fluka, 22183) dissolved in 4% PLLA mixture to make a final concentration of 4% cellulose. After drying, the cellulose covered loofah was dipped in 4% PLLA solution in chloroform, and finally coated with $HA \times 2.5$.

2.1.5. Loofah + PLLA + cellulose + HA

Loofah was coated with 4% PLLA solution in chloroform and dried prior to soaking in 4% cellulose (in 4% PLLA solution) and drying. It was finally coated with $HA \times 2.5$.

All scaffolds were sterilized at 90 °C with ethylene oxide prior to use in tissue cultures.

2.2. Biomechanical testing of scaffolds

The biomechanical tests of each loofah-based scaffold were carried out using the tensile testing machine (AG-I 10 kN, Shimadzu, Japan) at room temperature. The tensile tests were conducted with a constant

Table 1
Comparative ion concentrations of Tris-SBF and the Lac-SBF of this study [30,31].

Ion	Kokubo-SBF [mM]	Lac-SBFx1 [mM]	Lac-SBFx2.5 [mM]	Blood plasma	
				[mM]	meq/l
Na^+	142.0	142.0	142.0	142.0	142.0
Cl^-	147.8	103.0	103.3	103.0	103.0
HCO_3^-	4.2	27.0	27.0	27.0	27.0
K^+	5.0	5.0	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5	1.5	3.0
Ca^{2+}	2.5	2.5	6.25	2.5	5.0
HPO_4^{2-}	1.0	1.0	2.5	1.0	2.0
SO_4^{2-}	0.5	0.5	0.5	0.5	1.0
Lactate	–	22	26.5	[+] ion: [155 meq/l]	
Lactic acid [1 M]	–	36 ml	40 ml	[–] ion: [133 meq/l + 22 meq/l organic anions]	
Tris	50	–	–	–	–

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