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Fabrication and performance characteristics of tough hydrogel scaffolds based on biocompatible polymers

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ABSTRACT

Novel silane crosslinked tough hydrogel scaffolds were prepared using chitosan (CS) and polyvinyl alcohol (PVA) to give network structure and scaffolds properties. The influence of crosslinking and PVA concentration on scaffolds were studied. Fourier transform infrared spectroscopy (FTIR) spectroscopy confirmed the presence of incorporated components. Tensile strength (TS) and fracture strain analysis of scaffolds were detected owing to the mutual effect of chemically and physically crosslinked network. Tough hydrogel scaffolds having 90% CS and 10% PVA exhibited TS of 49.18 MPa and 10.15% elongation at break. The contact angle is less than 90° exhibited the hydrophilic nature of the scaffold. X-ray diffraction analysis (XRD) indicated the characteristics peaks fitting to CS and PVA and increase in the crystallinity of scaffolds. Cytotoxicity of scaffolds with different human fibroblast cell lines (F121, F192 and F84) for indirect method and human dermal fibroblast cell lines (F121) for direct method was evaluated. This indicated that these biomaterials were non-toxic, viable to the used cell lines, helpful in the growth of these cells and did not discharge toxic material damaging to the living cells.

1. Introduction

Hydrogels are employed as scaffolds materials for vehicles for drug delivery systems, tissue engineered scaffolds, biosensors for microfluidics, actuators for optics, and typical extracellular matrices for biological analysis. Tissue engineering is a multidisciplinary area in order to produce artificial tissues and organs such as: bones, skin, blood vessels and nerve conduits etc. [1–5]. Tissue engineering can be described as "rebuilding damage tissue" and can also be defined as to merge and culture cells into biodegradable material or scaffolds made of natural or synthetic materials and embedded in human body so, it can incorporate with host tissue and form an entirely efficient tissue in place of the defected tissue [6–8]. With the persistent aging of the inhabitants and less availability of the donor organs, the requirement for this technique is obvious. Tissue engineering technology has the capability to resolve this problem by repairing and regenerating the spoiled tissues [9,10]. The major challenge for tissue engineered scaffolds is to propose and manufacture customizable biocompatible and biodegradable materials that resemble the structural feature of extracellular matrices [11].

The scaffolds give support mechanically and transport cells to the specific repair spots. It provides assistance to control the task and formation of newly shaped tissue [12]. Scaffolds having properties of biodegradability, biocompatibility non-toxicity, and appropriate mechanical strength can be used for biomedical and tissue engineering applications. Natural and synthetic tissue scaffolds were prepared and modified for particular applications [13,14].

Polymer blending is used to produce new biomaterials showing arrangements of properties like mechanical strength, thermal and surface properties that could not be attained by individual polymers [15]. Natural and synthetic polymers have been considered for this reason. Synthetic polymers are easy to process and can modify the properties as a result of naturally arising metabolites in the human body. The commonly used synthetic polymers used in tissue engineering are polyvinyl alcohol (PVA), poly glutamic acid, polyurethanes, poly caprolactone, polyethylene glycol etc. PVA is a synthetic polymer and has been used for a lot of biomaterial applications due to water solubility, biodegradability, biocompatibility, easy to process, excellent mechanical properties

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and non-toxicity [16–22]. Natural polymers are extensively investigated due to their similarity with body tissues. Natural polymers such as starch, chitosan (CS), collagen [23], gelatin and albumin are used for many biomedical applications [24]. CS [poly-b(1-4)-d-glucosamine] has been commonly practiced as a biomaterial in drug delivery systems, tissue engineering, wound healing, and many other medical and biomedical uses [25–29]. It can be designed in a range of morphologies, such as hydrogel, gel, film, particles/nanoparticles, powder, sphere, and fiber/nanofibers [30–33]. CS is a naturally occurring polysaccharide and its mechanical properties can be improved by chemical modification and can give definite biological exchanges [15,34,35]. Natural and a synthetic polymer such as CS and PVA were integrated to produce an scaffold material with exceptional properties for tissue engineering such as; biocompatibility, biodegradability, must be capable to execute with appropriate host reactions, proper mechanical properties, as a carrier for growth aspect, role as extracellular matrix similarity, essential matrix/template for cell attachment, physical provision to direct the required diversity and increase of cells into the targeted useful tissue or part [3,36].

In this work, CS/PVA scaffolds were fabricated by dissolution casting process. The novelty of this work is the use of silane crosslinker i.e. tetraethoxysilane (TEOS) developing chemically and physically crosslinked networks for scaffolds materials properties. Structural, biomechanical, thermal and surface properties of CS/PVA scaffolds were observed by XRD, FTIR, biomechanical testing, differential scanning calorimetery (DSC) and contact angle analysis. TS, fracture strain and crack bridging by the established network of scaffolds was ascribed to the crosslinked network by TEOS. The in vitro cell viability of the scaffolds was estimated by indirect cytotoxicity assessment of the scaffolds with different human fibroblast cell lines. This analysis showed that these scaffolds are nontoxic to the existing cells.

2. Experimental

2.1. Materials

CS from crab shells with degree of deacetylation > 75%; Bulk density 0.15-0.30 g/cm^3; Viscosity 200-799 centipoise, polyvinyl alcohol (M_w: 85000–90000), acetic acid (99.7%), industrial methylated spirit, tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and TEOS were purchased from Sigma Aldrich (Milwaukee, WI). Ringer lactate was purchased from local chemist while simulated intestinal fluid (SIF) and simulated gastric fluid (SGF) were prepared using reported methods [30]. Phosphate buffer saline was also prepared by standard method. All chemicals were used as received without any purification.

2.2. Methods

Scaffolds were prepared by the following method: CS (5 wt% solution) in 0.5 M acetic acid was added in a glass reactor fitted with magnetic stirrer and mixed with PVA (5 wt% solution in deionized water) under constant stirring. Different weight ratios of CS/PVA were used (Table 1). When clear solution was obtained, TEOS (TEOS was dissolved in ethanol to get silanol) was added in each solution (5 wt% silanol). After 1 h, the resultant mixture was transferred into plastic container for drying at room temperature. To study the effect of the amount of crosslinker, TEOS (TEOS was dissolved in ethanol to get silanol) was added drop wise under constant stirring to different CS/PVA solutions (4, 8 and 12 wt% silanol) having ratio of 3/1 (Table 2). After drying, the scaffolds were vacuum dried at 60° C and stored in a desiccator. The compositions and codes of the scaffolds are shown in following Tables 1 and 2. The thickness of the scaffolds at various locations was determined by thickness meter and average value was evaluated.

Table 2
Codes and compositions of the scaffolds having varying amount of crosslinker.

Sample code	CSP4	CSP8	CSP12
Tetraethoxyorthosilicate (TEOS) (wt %)	4	8	12

Chitosan (CS): Polyvinyl alcohol (PVA) = 3: 1.
CSP = Blends of chitosan and polyvinyl alcohol.

2.3. Biodegradation of scaffolds

In order to explore the in vitro biodegradation, the different hydrogels scaffolds (25 mg) were placed in PBS solution for one, three and seven days. The scaffolds were removed and weighed after specific time. The decrease in weight was calculated by subtracting final weight (after degradation) from initial dried weight of the scaffold.

2.4. Swelling analysis

Swelling analysis in ringer lactate solutions, SIF, and SGF was done. In this analysis, the swelling was determined by placing different scaffolds (35 mg) in ringer lactate, SIF and SGF for different time intervals. The specimens were weighed after removing their respective solvent at specific time intervals. The swelling ratio was calculated by following equation.

$$\text{Swellingratio} = (Ws - Wd)/Wd$$

Where, Ws is the swollen weight and Wd is the dried weight of the scaffolds.

2.5. FTIR analysis

FTIR was used to analyze the existence of specific functional groups in the scaffolds using FTIR spectrophotometer (Nicolet, 6700) from Thermo Electron Corporation, USA. The spectra were taken over the range of 4000–500 cm^{-1} at resolution of 6.0 cm^{-1} with average of 200 scans. Attenuated total reflectance (ATR) mode with diamond crystal was used. FTIR spectra of pure PVA and CS were also attained for comparison with the scaffolds.

2.6. XRD

The structural analysis of scaffolds was performed on STOE STADI P power diffractometer with (Cu) Kα_1 radiation with wave-

Table 1
Codes and compositions of the scaffolds having varying amount of polyvinyl alcohol (PVA).

Scaffold	CSP9010	CSP8020	CSP7030	CSP6040	CSP5050	CSP4060	CSP3070	CSP2080	CSP1090
Chitosan (CS) (Wt %)	90	80	70	60	50	40	30	20	10
Polyvinyl alcohol (PVA)(Wt %)	10	20	30	40	50	60	70	80	90

Chitosan (CS) solution = 5 wt %, Polyvinyl alcohol (PVA) solution = 5 wt %, Tetraethoxyorthosilicate (TEOS) = 5Wt %.
CSP = Blends of chitosan and polyvinyl alcohol.