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The innovative combined microwave-assisted and photo-polymerization technique for synthesis of the novel degradable hydroxyethyl (meth) acrylate/gelatin based scaffolds

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ABSTRACT

The discovery of novel biodegradable biomaterials able to support and control cellular activity as well as development of an enhanced and efficient method for their fabrication, are of paramount importance in the field of tissue engineering. This study highlights the design of novel degradable hydrogels based on gelatin and hydroxyethyl (meth)acrylates using the innovative combined two-step sequential microwave-assisted and UV photo-polymerization technique. Chemical composition, morphology, swelling capacity and degradation rate of the synthesized hydrogels were evaluated by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), swelling and weight loss measurements. As an initial step for evaluation of performance of the hydrogels in the biological environment, the *in vitro* biocompatibility of these hydrogels, was evaluated using L929 mouse fibroblasts. Obtained results demonstrated that the hydrogels possess a porous morphology with interconnected pores, 50% *in vitro* degradation after 7 months, and satisfied biocompatibility on L929 fibroblast cells. These unique performances of the hydrogels make them promising candidates for *in vivo* evaluation in clinical studies aiming at tissue regeneration.

1. Introduction

Designing of novel high performance bioinstructive scaffolds, as well as development of novel methods for their fabrication, are the key factors for rapid progress in the field of tissue engineering. Hydrogels are attractive scaffolding materials owing to their unique compositional and structural similarities to the natural extracellular matrix, efficient mass transfer and their desirable ability for cellular proliferation and survival [1,2]. Recently, gelatin based hydrogels which are offered excellent bio-affinity and induced the regeneration of tissue are promising candidates in tissue engineering [3].

Polymeric scaffolds for tissue engineering can be prepared with a multitude of different techniques including electrospinning, phase-separation, freeze drying, 3D bioprinting, microwave-assisted and photo-polymerization [4,5]. Nevertheless, one of the major trends in the field of tissue engineering is the development of an enhanced and efficient fabrication scaffolds technique. The novelty of this study is the new approach to the design of the biocompatible and degradable hydrogels by an enhanced and for first time used, combined microwave-assisted and UV photo-polymerization technique. Hydroxyethyl (meth)acrylates are attractive scaffolding components due to their high water content, biocompatibility and wide biomedical applications [6]. Gelatin is selected as it is a low cost adhesive, biocompatible protein produced by acidic or basic degradation of collagen [7]. The hydrogels were successfully synthesized by the novel combined technique and their chemical structure, morphology, swelling behavior, and degradation rate were analyzed. For the biocompatibility assessment of the hydrogels, proliferation of L929 fibroblast cell measured by MTT method was used. Obtained findings suggest efficiency of the proposed multicomponent, biodegradable, and biocompatible hydrogels as promising tunable archetype for scaffold application.

2. Materials and methods

The three series of novel hydrogels based on 2-hydroxyethyl acrylate (HEA, Sigma-Aldrich), 2-hydroxyethyl methacrylate (HEMA, Sigma-Aldrich) and gelatin (G, Sigma-Aldrich) were prepared by the innovative combined two-step sequential

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microwave-assisted (MW) and UV photo-polymerization process (Fig. 1(a)). In the first step, a hydrogels composed of hydroxyethyl (meth)acrylates were formed by microwave (Supplementary Table 1). In the second step these rigid and brittle porous structures were subsequently poured with aqueous solution of components HEA/G and HEMA/G and then the polymerization was achieved by UV photo-irradiation (Supplementary Table 2).

Ethylene glycol dimethacrylate (EGDMA, Aldrich), potassium persulfate (KPS, Fluka) and *N,N,N′,N′*-tetramethylene diamine (TEMED, Aldrich) were used as a crosslinking agent, initiator and activator, respectively. Benzoin (Aldrich) was used as photoinitiator. The mole fraction of gelatin in the samples were varied (0.0, 5.0, 7.0 and 10.0%).

Fourier transform infrared spectrometer (BOMEM Michelfan MB-102 FTIR) was used to analyze the chemical composition of the hydrogels. The scanning electron microscope (JEOL JSM-5800 LV) was used to observe specimen's morphology. The swelling performances of the hydrogels were evaluated in the phosphate buffer solution (pH 7.40, 37 °C). The amount of fluid absorbed as a function of time was monitored gravimetrically [8].

In order to investigate the *in vitro* degradation rate, the xerogels were incubated in a tube containing 10 ml phosphate buffer (pH 7.40 at 37 °C) during 7 months. After incubation, samples were removed from the media, washed with distilled water several times to remove the excess salts, and oven-dried to constant mass. Mass loss was calculated by comparing the initial mass (W_o) with the mass measured at a given time point (W_t), as shown in the following equation [9]:

$$Mass\ loss = \frac{(W_0 - W_t)}{W_0} \times 100\% \quad (1)$$

To evaluate the biocompatibility of the novel hydrogels, L929 fibroblast cells were cultured on a 96-well plate. The hydrogels were cultivated in the cell culture media during 24 h. Cells were exposed to the prepared supernatants during 24 h and measuring the cell viability/proliferation, MTT method was utilized [10].

3. Results and discussion

The presented FTIR spectra (Fig. 1(b)) revealed the characteristic ester C=O stretching peaks at 1730 cm^{-1}, two moderate peaks in the range of 1000 to 1300 cm^{-1}, which correspond to ester C—O stretchings, the peak of OH group at 3435 cm^{-1}, and aliphatic peaks in the range of 2900–3000 cm^{-1} for all samples. Obtained FTIR spectra highly indicate successful pathway of scaffold synthesis. Incorporated G is not clearly confirmed by FTIR spectra due to characteristic peaks for G are overlapped by polymer peaks (moderate right scholder around 1650 cm^{-1} at strong C=O band of polymer can be attributed to G from PHEMA/P(HEMAG10)).

The morphology of scaffold influences the attachment of cells to its surface [11]. Additionally, one of the requirements for hydrogels to attach cells is to easily deliver oxygen, nutrients, water soluble

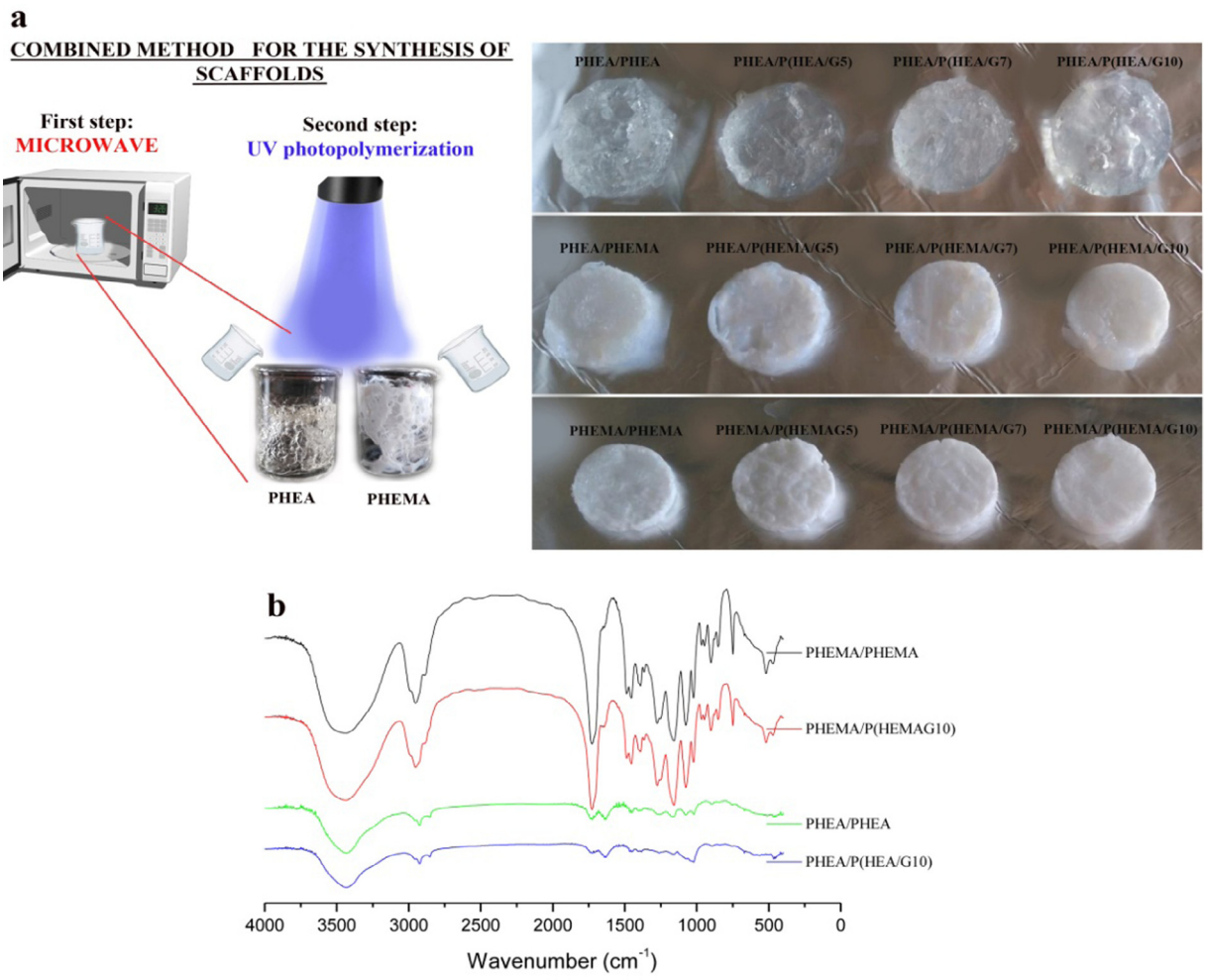

Fig. 1. (a) Synthesis route of the scaffolds (b) FTIR spectra.