Binary phase solid-state photopolymerization of acrylates: design, characterization and biomineralization of 3D scaffolds for tissue engineering

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ABSTRACT: Porous polymer scaffolds designed by the cryogel method are attractive materials for a range of tissue engineering applications. However, the use of toxic cross-linker for retaining the pore structure limits their clinical applications. In this research, acrylates (HEA/PEGDA, HEMA/PEGDA and PEGDA) were used in the low-temperature solid-state photopolymerization to produce porous scaffolds with good structural retention. The morphology, pore diameter, mineral deposition and water absorption of the scaffold were characterized by SEM and water absorption test respectively. Elemental analysis and cytotoxicity of the biomineralized scaffold were revealed by using XRD and MTT assay test. The PEGDA-derived scaffold showed good water absorption ability and a higher degree of porosity with larger pore size compared to others. XRD patterns and IR results confirmed the formation of hydroxyapatite crystals from an alternative soaking process. The overall cell proliferation was excellent, where PEGDA-derived scaffold had the highest and the most uniform cell growth, while HEMA/PEGDA scaffold showed the least. These results suggest that the cell proliferation and adhesion are directly proportional to the pore size, the shape and the porosity of scaffolds.

KEYWORDS: binary phase solid-state photopolymerization; phase separation; tissue engineering; biomineralization; MTT

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1 Introduction

Porous scaffolds have attracted significant interest in the field of tissue engineering for developing biological replacements for restoration or regeneration of damaged tissues [1–2]. Regeneration or growth of tissues typically depends on the cell and its scaffold [3]. Therefore, scaffolds are designed principally to facilitate cell colonization and formation of tissue for imitating the tissue extracellular matrix (ECM) [3–4]. An ideal scaffold for tissue engineering applications should be compatible with microstructure and physicochemical properties. The fabrication of the scaffold must take into account the tissue structure, in which it will be implanted [5]. A perfect scaffold must be biocompatible, endure regular shape, having desired mechanical strength and highly interconnected pores for cell growth and colonization [1,6].

A wide range of methods has been used to produce porous scaffold for tissue engineering. These include foaming, emulsionification, solvent casting, particle/salt leaching, freeze drying, thermally induced phase separation, electro-spinning, hydrogel and cryogel [4,7–13].

Among them, the cryogel method is one of the most innovative techniques, which produces highly porous and well-interconnected structure [14–15]. Different types of porous materials such as aligned and hybrid scaffolds have been successfully prepared by using the same technique [14,16–19].

The cryogel method uses monomers dissolved in water to form a solution. A thermodynamic flux is created within the system by lowering its temperature to subzero level, in order to trap the monomers in the solvent phase [20–21]. Cross-linking then can be achieved by the thermal or chemical methods [22–23]. Finally, the solvent is removed through sublimation under vacuum by producing voids in previously ice occupied regions [21,24]. A major drawback of this method is that during sublimation numerous loosely cross-linked monomers can re-dissolve in a solvent causing collapse and destruction of the pores formed by the frozen solvent. To overcome this problem and to maintain the porous structure, binding materials such as glutaraldehyde is commonly used to increase its structural stability [25–27]. These types of additives are highly toxic and may have an adverse effect on the final structure and application of porous materials due to change in pH [22,28–29]. Hence, we have proposed a facile and green method to produce porous scaffolds.

Herein, a novel method is proposed for binary phase solid-state photopolymerization in which the aqueous solutions of acrylates were photopolymerized at completely frozen state below their eutectic point. At this point, both phases (solvent and solute) get frozen, and phase separation takes place resulting in the formation of the three-dimensional (3D) interconnected network by the solvent. Due to the frozen state of both phases, there is no need for other toxic binding materials for gelation. In addition, freezing of the solution causes separation of binary phase that results in extraordinary smoothening of the pore walls, enhances the cell viability and cell growth for tissue engineering application [30–32]. To our best knowledge, it is a novel method and has not been reported previously for porous scaffolds. Here 2-hydroxyethyl acrylate (HEA), 2-hydroxyethyl methacrylate (HEMA) and poly (ethylene glycol) diacrylate (PEGDA) monomers (Scheme 1) were used for polymerization on the basis of their solubility and applications in tissue engineering [33–37].

2 Experimental

2.1 Materials

HEA, HEMA and PEGDA (MW = 400) were purchased from Aladdin Industrial Corporation. 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methyl-propiophe (photoinitiator 2959) was donated by Tianjin Juri New Materials Company. Mouse fibroblasts (L929) were offered by Department of Microbiology, Peking University Health Science Center. Other chemical agents were obtained from China National Medicines Corporation Ltd. and used as received.

2.2 The fabrication of porous polymer scaffold

In the fabrication of porous scaffolds, HEA/PEGDA, HEMA/PEGDA and PEGDA and water (as a solvent) were