Research paper

Lactone-layered double hydroxide networks: Towards self-assembled bioscaffolds

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ABSTRACT

This paper describes the conversion of a layered anionic initiator (carbonate-intercalated layered double hydroxide, (LDH-carbonate)) into a self-assembled resin-embedded network during the in-situ polymerization of one or more lactone monomers using the LDH-carbonate as the sole initiator. Uniquely in this paper, no long-chain acid intercalant is present in the LDH-carbonate to act as an additional initiator species, and this is the first known report of a copolymerisation of these lactones using LDH as an initiator. The formation of a network is in marked contrast to the behavior of most in-situ polymerisations using layered species, where the latter retains its layered structure at the molecular level and is either intercalated or exfoliated to form a nanocomposite. The molecular disintegration of the LDH sheets is unusual. Nine new insoluble materials (scaffolds) are isolated from various L,D-lactide & ε-caprolactone (LC) and L,D-lactide & δ-valerolactone (LV) copolymer hybrids. The latter hybrids are polymerised using the LDH-carbonate as initiator at 150 °C for 24 h without using conventional metal catalysts. Each insoluble phase is isolated from each primary hybrid product using dichloromethane (DCM) to selectively dissolve the soluble polymer phase.

X-ray diffraction (XRD) is used to verify the morphology of the insoluble phases. This demonstrates that the molecular sheets of the LDH-carbonate are fully dismantled during the polymerization. Porous, network morphology is established for some of the insoluble phase structures using scanning electron microscopy (SEM). This indicates potential suitability of these self-assembled insoluble phase materials as bioscaffolds for artificial cell growth. Nuclear magnetic resonance spectrometry (NMR) was used to determine the ratio of ester to acidic carbonyls in the insoluble phase. Energy Dispersive X-ray spectroscopy (EDX) was also used to determine the ratio of magnesium to aluminium in the insoluble phases.

1. Introduction

Tissue engineering has evolved greatly since the mid-1980s into a multidisciplinary field targeting the restoration, maintenance and improvement of tissue functions Faÿ et al. (2007), Langer and Vacanti (1993), Chen et al. (2009), Vaz et al. (2005), Vunjak-Novakovic et al. (2010), Oberpenning et al. (1999), Schmidt and Leach (2003), Kuo et al. (2010). In particular, poly(lactic acid) (PLA) is a popular polyester material that is used in tissue engineering because of its excellent biocompatibility and mechanical properties. Good adherence and differentiation properties have been observed for osteoblasts cultured on PLA membranes, Santos et al. (2009), Liu et al. (2004). Similar to PLA, poly(ε-caprolactone) (PCL) and poly(δ-valerolactone) (PVL) are often employed for use in bio-scaffold applications. Their advantage in this application is their relatively low degradation rate (during hydrolysis in aqueous media). Therefore, they have been copolymerized with a variety of polymers including collagen, poly(glycolic acid), poly(lactic acid) and poly(ethylene oxide), Vroman and Tighzert (2009).

Layered double hydroxides (LDHs) comprise an unusual class of layered inorganic materials with positively charged layers and weakly bound, usually exchangeable, charge-balancing anions Manzi-Nshuti et al. (2009). LDHs have been studied and characterised by multiple researchers, e.g., Pérez-Amaro et al. (2009), Swanson et al. (2013) and Kang et al. (2004).

Ring-opening polymerization (ROP) has been proposed as the main mechanism by which a monomer can be ring-opened at the cation of an LDH sheet, and can then propagate to form a polymer chain anchored on this LDH sheet, McCarthy et al. (2013). Generally, ROP may occur by either cationic, anionic or coordination-insertion routes, Krichedorf (2001). In addition to ROP at the LDH sheet, a combination of polycondensation of ring-opened monomers and ROP by free ions in the polymer bulk may also occur.

In this paper, the formation of various insoluble polymer-based Mg poly(lactone) networks have been demonstrated by polymerizing different lactone monomer combinations using LDH-carbonate as an initiator for the first time; this is an approach, which avoids the use of any potentially toxic conventional metal catalysts. There is also no long-chain acid intercalant present in the LDH, as used previously (McCarthy et al., 2013), which proves that the LDH itself is sufficient to initiate polymerization and network formation without the aid of a long chain intercalant. Some of the insoluble network phases are potentially suitable for use as bioscaffolds for cell growth, based on their porous morphology. The main advantage of this system over established scaffold fabrication technologies is the capacity to tailor the chemistry of the resin material using different monomer combinations and types of layered double hydroxide. The scaffold is self-assembled at the molecular level and does not require specialised electrospinning or other physical deposition technologies (e.g. chemical vapour deposition, photolithography, or electron beam lithography, Prabhakaran et al. (2012). This provides the potential to remove such deposition steps from scaffold preparation, hence reducing cost. It also provides the potential to modify the cations and intercalated species of the LDH-initiator to include beneficial active ingredients for the cell growth process directly in the scaffold structure.

2. Materials and methods

ε-caprolactone (97%) and δ-valerolactone (technical grade) were both obtained from Aldrich. ε-caprolactone, which is liquid at room temperature, and δ-valerolactone were stored at 4 °C before use. L,D-lactide (99%) was obtained from Alfa Aesar. All the three materials mentioned above were used as monomers in the polymerization process. The initiator, synthetic layered double hydroxide carbonate, was obtained from Aldrich. LDH-carbonate has a layered structure with a high anionic exchange capacity that allows it to host and release various anionic compounds. Its chemical formula is $Mg_6Al_2(CO_3)\cdot(OH)_{16}\cdot4H_2O$. Since L,D-lactide and LDH-carbonate are both sensitive to moisture, it is essential to avoid their re-adsorption of water. Thus, when they were not being used immediately, they were both stored over a desiccant. L,D-lactide was sublimed immediately prior to reaction to remove moisture.

2.1. Sample preparation

A schematic of the specimen preparation process is given in Fig. 1. This comprised the polymerization of a mixture of monomer(s) plus LDH followed by dissolution of the product in methylene chloride followed by centrifugation of the solution to result in an insoluble residue which was dried to result in two separate phases of the polymer, soluble (SOL) and insoluble (INSOL).

2.2. Reaction set 1: Mixture of L,D-lactide and LDH-carbonate

A mixture of L,D-lactide and LDH-carbonate was prepared in the ratio of 95:5 by mass. 5 g of the mixture was put into a 100 ml glass bottle, followed by intense mixing using a mechanical mixer (Vortex Genie obtained from VWR International). Since L,D-lactide sublimes at

125 °C, an aluminium foil was used to seal the vial and retain the subliming monomer at the reaction temperature (150 °C). The product of this reaction was labelled PLDLA-HYB (HYB = hybrid), while its soluble and insoluble phases were labelled PLDLA-SOL and PLDLA-INSOL, respectively.

2.3. Reaction set 2: Mixtures of L,D-lactide & ε-caprolactone (LC) and L,D-lactide & δ-Valerolactone (LV) with LDH-carbonate

The LDH-carbonate initiator comprised 5% by mass of each overall reaction mixture, while the monomer mixtures, L,D-lactide & ε-caprolactone (hereafter referred to as LC) and L,D-lactide & δ-valerolactone (hereafter referred to as LV) were mixed in the ratios of 1:2, 1:1, and 2:1, respectively, by mass. The resulting products were LC1:2-HYB, LC1:1-HYB, LC2:1-HYB, LV1:2-HYB, LV1:1-HYB, and LV2:1-HYB.

2.4. Hybrid polymerization process

All the initial hybrid polymer products with different initial monomer ratios were synthesised at 150 °C for 24 h in a Heraeus Incubator oven. The 100 ml reaction vials were fully sealed by aluminium foil wrap at the top of each vial to prevent escape of monomer vapour at temperatures > 125 °C. A minimal headspace was allowed to ensure that the L,D-lactide vapour remained trapped in intimate contact with the reaction bulk.

Separation of each initial hybrid product into soluble (SOL) and insoluble (SOL) fractions by methylene chloride solvent extraction and centrifugation. Each initial hybrid product consisted of a soluble polymer, an insoluble polymer-salt complex phase and any residual monomers. Therefore, methylene chloride (dichloromethane (DCM)) (obtained from Fisher Chemicals) was used as a solvent to dissolve the soluble polymer phase so that the insoluble phase could be isolated. The extraction procedure was as follows: Once each reaction sample was cooled to room temperature, 60 ml DCM was added to the sample in a vial and the solution was mixed with a magnetic stirrer for 12 h (the concentration of the solution was about 0.083 g/ml). Once the hybrid was fully dissolved in the solvent, the solution was equally separated into four 15 ml centrifuge tubes that were placed in a centrifuge. The centrifuge (Sigma 4-16 centrifuge) was operated at a speed of 800 rpm or 10,375 RCF (Relative Centrifuge Force), for 10 min. When the centrifugation was finished, the upper supernatant was gently poured off or removed by syringe, and the insoluble residue was allowed to dry fully at room temperature for 24 h. Then, the centrifuge tubes were placed in an oven at 50 °C for an hour to ensure complete removal of the methylene chloride (boiling point: 39 °C).

2.5. Characterization methods

The samples used for characterization studies were desiccated and finely powdered. Thermogravimetric analysis (TGA) was performed using a Perkin-Elmer/Seiko machine under a nitrogen atmosphere avoiding further oxidations. Isothermal thermogravimetry was performed by heating the well-mixed primary product after polymerization at 150 °C for 6 h with an initial heating rate of 20 K/min. Note that these isothermal TG measurements were unable to remove the δ-
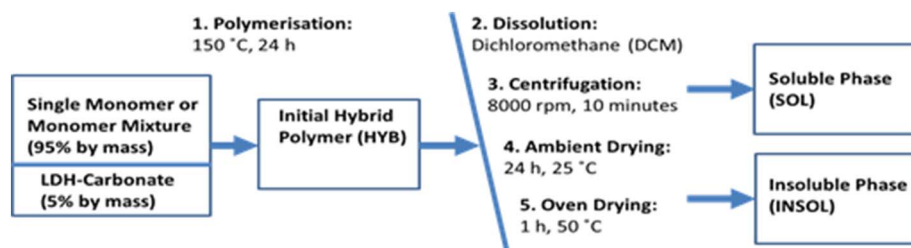

Fig. 1. Process schematic for polymerization of various products and their extraction with methylene chloride to form scaffolds.