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Porous amphiphilic biogel from facile chemo-biosynthetic route

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Abstract: Grafting of medium chain length poly-3-hydroxyalkanoates (mcl-PHA) with glycerol 1,3-diglycerolate diacylate (GDD) in acetone was performed using benzoyl peroxide as initiator. A detail mechanism scheme provides significant improvement to previous literature. Radical-mediated grafting generated carbon inter-linking of mcl-PHA and GDD resulted in macromolecular structure with gel properties. Thermal properties of the copolymer for different graft yields were investigated as a function of initiator concentration, GDD monomer concentration, incubation period and temperature. The water absorption and porosity of the gel were significantly improved relative to neat mcl-PHA.

Keywords: biogel; chemo-biosynthetic; biopolymer; radical grafting

INTRODUCTION

Polyhydroxyalkanoates (PHA) are well-known biopolymers with attractive biocompatibility. They are accumulated within certain bacterial species in the form of granules when the microorganisms experience imbalanced growth conditions viz. simultaneous excess carbon source and limitation of nutrients such as nitrogen and phosphorus. Two categories of PHA can be differentiated i.e. short-chain-length polyhydroxyalkanoates or scl-PHA, comprising of monomers with four- to five carbon atom length, and medium-chain-length polyhydroxyalkanoates or mcl-PHA, made up of 6- to 14- carbon atom length monomers. Modification and functionalization of PHA, intended for tuning the features, are important for certain applications. Functionalization of PHA on the side chain, for example, can alter the polymer interaction behaviour by introducing elements of hydrophilicity.
One of the functionalization techniques is grafting. Graft copolymerization of PHA forms a modified segmented copolymer with interesting properties, particularly in terms of wettability and thermo-mechanical strength. The grafting processes can be carried out in several ways, including chemical, radiation, and plasma discharge methods. The current grafting methods for many polymers are equally applicable in the case of PHA functionalization. For example, the benzoyl peroxide-initiated graft polymerization of 2-hydroxyethylmethacrylate (HEMA) onto poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) enhances the crystallinity and wettability of the biopolymer.

Grafting applies radical intermediates during the reaction, which mediate the polymerization of a vinyl monomer as grafted branches on the main polymer chain through ‘grafting onto’, ‘grafting through’ and ‘grafting from’ techniques. Free radical grafting via radical initiators is widely used for the modification of polymers. Benzoyl peroxide is one of the initiators extensively used to functionalize bacterial polyesters. It is a simple and robust method. Benzoyl peroxide has been reported to be more efficient compared to other common thermal initiators, such as azo-bis-isobutyronitrile (AIBN) and other peroxyl initiators. When the concentration of the propagating radical balances the rate of radical termination, polymers with high number average molecular weight and low dispersity can be obtained. The catalyst determines the equilibrium constant between the active and dormant species, which in turn determine the polymerization rate.

In this study, medium-chain-length poly-3-hydroxyalkanoates (mcl-PHA), obtained from bacterial fermentation, was graft copolymerized with glycerol 1,3-diglycerolate diacylate (GDD) by free radical polymerization. The grafted product, PHA-g-GDD, was prepared through thermal incubation process with all reaction components mixed together in a selected organic solvent. The effects of different initiator concentrations, in this case benzoyl peroxide (BPO), were investigated alongside incubation temperature and time, and initial GDD concentration. The PHA-g-GDD copolymer was characterized and mechanism of the grafting reaction was proposed.

EXPERIMENTAL

Materials

Lauric acid (C_{12}H_{24}O_{2}, M_{w} 200.23, CAS, 143-07-7) for synthesis was purchased from Merck as a sole carbon source in fermentation medium to produce medium-chain-length poly-3-hydroxyalkanoates (mcl-PHA) by Pseudomonas putida Bet001. The mcl-PHA was obtained by solvent extraction and purified through repeated methanol precipitation and washing steps.

Benzoyl peroxide (BPO) (C_{4}H_{10}O_{4}, M_{w} 242.23, CAS 94-36-0), (with 25 % H_{2}O, used as received) for synthesis was purchased from Merck Millipore (Darmstadt, Germany) and applied as a sole radical initiator of the grafting reaction. Glycerol 1,3-diglycerolate diacylate (GDD) (C_{15}H_{24}O_{9}, M_{w} 348.35, CAS 60453-84-1) was purchased from Sigma-Aldrich (Saint Louis, USA) and used as received as well.
Radical Grafting of Mcl-PHA

Monomer Composition of mcl-PHA

The monomer content of the mcl-PHA was determined by gas chromatography. Sufficient amount of the sample was subjected to methanalysis. Two milliliters of dichloromethane (DCM) and 2 ml mixture of methanol and sulphuric acid (1:1 volume ratio) were added to the sample. The mixture was incubated at 100 °C for 2 hours and 20 minutes in a heating block. Distilled water was added, and the mixture vortexed for about one minute before left standing overnight or at least four hours for phase separation. The organic layer at the bottom fraction containing the methylated products was transferred into a vial and mixed with sodium sulphate ($\text{Na}_2\text{SO}_4$) to remove excess water. The organic layer was auto-injected into a fused silica capillary column (30 m length × 0.32 mm internal diameter × 0.25 μm film) (Supelco SPB™-1, Bellefonte, Pennsylvania, USA) fitted within a gas chromatography machine (Trace GC Ultra: Thermo Scientific, Rodano, Milan, Italy) with flame ionization detector. During the process, helium was used as a carrier gas at the rate of 48.3 ml min⁻¹ and 0.41 bar pressure. Four types of monomers, i.e. 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD), and 3-hydroxydodecanoate (3HDD), at 4 mol%, 37 mol%, 38 mol%, 21 mol%, respectively, hence, alternatively known as $\text{P(3HO-co-3HHx-co-3HD-co-3HDD)}$ were identified and quantified in the mcl-PHA samples.

Preparation of PHA-g-GDD Copolymers

Copolymers were prepared from incubation of 50 g dm⁻³ mcl-PHA and 0.14 mol dm⁻³ GDD in acetone at varying concentrations of BPO. Oxygen was purged out of the solution with nitrogen gas for ten minutes and the vial was subsequently capped to introduce airtight conditions. It was incubated within a heating block at 70 °C and 90 °C. The mixture was left to cool to ambient temperature (25 ± 1 °C) post-incubation before adding methanol to allow precipitation of the gel product, and separate the non-grafted GDD monomer and GDD homooligomers in the soluble fraction at the same time. Successful grafting was indicated by the increase in the mass of precipitated product over the initial mass of mcl-PHA used, calculated as follows:

$$\text{Graft yield} = \frac{W_f - W_i}{W_i} \times 100$$

where $W_f$ is the final mass of grafted PHA after reaction, and $W_i$ is the initial mass of PHA before reaction.

Characterization of PHA-g-GDD Copolymers

Fourier Transform Infrared-Attenuated Total Reflectance (FTIR-ATR) Spectroscopy

FTIR-ATR was used to record the spectra on Perkin-Elmer Spectrum 400 FT-IR and FT-NIR Spectrometer (Perkin-Elmer Inc., Wellesley, MA, USA), equipped with PIKE GladiATR hovering monolithic diamond ATR accessory (Pike Technologies Inc., USA) at room temperature. The samples were placed on the monolithic diamond ATR probe and fastened against the diamond crystal plate using force adaptor. Spectra were recorded between 4000 and 450 cm⁻¹ using cuts of 0.5×0.5 cm films.

Simultaneous thermal analysis (STA) and differential scanning calorimetry (DSC)

The applied thermal analysis was destructive Simultaneous Thermal Analysis (STA) of ASTM-E2550-11 thermal stability method. The machine used was Perkin Elmer STA 6000 (Perkin-Elmer Inc., Wellesley, MA, USA) running on tandem differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The sample was prepared in the
form of film. The analysis was programmed to initialize from 30 °C until 800 °C at a rate of 10 °C min\(^{-1}\) under a nitrogen gas stream of 10 ml min\(^{-1}\).

Proton nuclear magnetic resonance (\(^1\)H NMR)

Sufficient quantity of grafted sample was washed with deuterated chloroform (CDCl\(_3\)). The solvent was filtered to separate the undissolved component using borosilicate glass syringe equipped with 0.22 μm polytetrafluoroethylene (PTFE) disposable filter (11807–25; Sartorius Stedim, Goettingen, Germany). The filtrate fraction with dissolved component was subjected to NMR analysis. The spectrum was acquired using a JEOL JNM-GSX 270 FT-NMR spectrometer (JOEL, Tokyo, Japan) at 400 MHz against tetramethylsilane (TMS) as internal reference. For mcl-PHA, the sample was simply dissolved in CDCl\(_3\) and filtered.

Carbon solid-state nuclear magnetic resonance (\(^{13}\)C NMR)

Sufficient quantity of undissolved grafted sample was washed with chloroform to remove residue, dried and powderized for solid-state analysis. The spectrum was acquired using a JEOL JNM-ECX500 NMR spectrometer (JOEL, Tokyo, Japan) at 500 MHz.

Water absorption and porosity studies

The samples were cut into small cubes with measured height, length and breadth, and then immersed in deionized water overnight. For porosity study, the cubes were immersed in 95 % ethanol solution for an hour before being left overnight immersed in deionized water.\(^{11,20,22}\) Gravimetric measurement was used to record the weight change for each of the samples in order to determine the degree of swelling from water absorption (Eq. 2). To calculate the porosity of the samples (Eq. 3), solvent replacement method was used. The calculations involved are as follows:

\[
\text{Degree of swelling} = \frac{W_w - W_d}{W_d} \times 100 \quad (2)
\]

\[
\text{Porosity} = \frac{W_w - W_d}{\rho V} \quad (3)
\]

where \(W_d\) is the weight of the sample before immersing (dry), \(W_w\) is the weight of the sample after immersing (wet), \(V\) is the volume of the sample and \(\rho\) is the density of the solvent used, in this case, denatured 95 % ethanol, which is 790 kg m\(^{-3}\).

RESULTS AND DISCUSSION

Characterisation of PHA-g-GDD copolymers

FTIR-ATR Spectroscopy

Ester vibrations were detected for both individual mcl-PHA and GDD samples. The carbonyl absorptions were observed at 1726 cm\(^{-1}\) and 1718 cm\(^{-1}\), respectively, while the corresponding CO bond signals appeared at 1162 cm\(^{-1}\) and 1189 cm\(^{-1}\). Meanwhile, the vibration signaling wavelength for symmetric \(-\text{CH}_2-\) of the samples were observed at 2858 – 2855 cm\(^{-1}\), and 2926 – 2925 cm\(^{-1}\) for asymmetric \(-\text{CH}_3\). No asymmetric \(-\text{CH}_3\) signaling was detected in pure GDD samples (Fig. 1).
Fig. 1. FTIR spectra of grafted materials obtained from reaction mixture at 70 °C (left) and 90 °C (right). A & G neat mcl-PHA; B & H PHA-g-GDD with $3.0 \times 10^{-6}$ mol dm$^{-3}$ BPO; C & I PHA-g-GDD with $5.0 \times 10^{-6}$ mol dm$^{-3}$ BPO; D & J PHA-g-GDD with $10^{-5}$ mol dm$^{-3}$ BPO; E & K PHA-g-GDD with $1.5 \times 10^{-5}$ mol dm$^{-3}$ BPO; and F & L GDD monomer.

In grafted copolymer, signal from the presence of hydroxyl group introduced by the GDD monomers was evident as the copolymers exhibited new broad signals of $\equiv$OH group shifted to 3432 – 3431 cm$^{-1}$. In addition, a strong shift at about 1151 cm$^{-1}$ signifying ester bond signal, available in both mcl-PHA and GDD monomer, was present in abundance for the grafted product samples compared to the neat mcl-PHA samples. Ether bond presents exclusively in pure GDD samples was also evidenced with strong signal at about 1091 cm$^{-1}$. It can be concluded that grafting of mcl-PHA with GDD was successful.

$^1$H NMR

A representative $^1$H NMR spectrum for the grafted products is shown in Figure 2. The spectrum shows all typical signals for neat PHA. The signal indicating a successful grafting process was found between 1.8 ppm to 1.9 ppm. The signal was assigned to overlapping signals of methine and methylene hydrogen atoms (-CH-) and (-CH$_2$-) on the backbone of polyacrylate, labelled as h and i. The peak also signifies the hydrogen atoms at the grafting position of PHA, reflecting α and β positions to the carbonyl of the terminal fatty acid of
PHA, labelled as $f$ and $g$ in Figure 2, respectively. Additionally, another new signal of interest was evident between 3.7 ppm to 3.8 ppm. This is associated with hydrogens from methylene and methine groups of the triglycerol core of GDD, labelled as $j$ and $k$.

A broad signal, overlapping with the chloroform peak at 7.3 ppm (labelled as $z$), most likely indicates the presence of a benzene ring, originating from the phenyl radical, which is generated by the initiator BPO and attaches to the very first monomer during the polymerization process.

Extensive cross-linking in the copolymer, due to the divalent structure of GDD, leads to a gel-like material, which is insoluble in solvent applied for recovery and purification in this study *i.e.* methanol. However, some of the grafted mcl-PHA copolymer could be dissolved in hydrophobic solvent such as chloroform, and subsequently investigated for NMR studies. This separated component represents fraction of the grafted copolymer with relatively lower molecular weight from less extensive cross-linking, thereby providing an organic solvent-soluble material for structural authentication.
$^{13}$C NMR

Solid-state $^{13}$C NMR authentication was also performed since major fraction of the grafting product exhibited gel-like morphology, which is virtually insoluble in any organic solvent. However, the signal resolution was rather poor with several combined peaks especially related to aliphatic components of both mcl-PHA and GDD. Nevertheless, additional signal of minute amount of carbonyl ketone groups was observed around 210 ppm on the spectrum (see Supplementary Materials, Fig. S5), indicating oxidation of secondary alcohol hydroxyl group within GDD, occurring as a side reaction due to the cascading radical reaction environment. Elaboration on the spectrum signals is made available in the Supplementary Materials.

**Mechanism of mcl-PHA grafting with GDD**

From structural studies, a detail grafting reaction mechanism was proposed, which presented a significant improvement to previous literature.$^{11, 13}$ While the proposed reaction scheme still follows a typical three-step radical polymerization, which includes initiation, propagation and termination phases, it introduces a thorough revision of participating reactive components in the reaction.

Firstly, the grafting process involving mcl-PHA requires an unsaturated terminal monomer unit, referring to a double bond between the $\alpha$- and $\beta$-carbon, most likely originating from thermal degradation of mcl-PHA. There are several possibilities to introduce the double bond via elimination processes, as corroborated by other similar studies$^{23, 24}$ and shown in Figure 3.

Grafting of mcl-PHA with GDD to obtain PHA-g-GDD copolymer starts with the dissociation of BPO into a benzene radical. It is proposed that the radicals start off the initiation step by attacking the $\beta$-carbon of the double bond to introduce a radical on the $\alpha$-carbon.

Fig. 3. Possible routes of mcl-PHA degradation that contribute to alkenyl end group able to participate in grafting reaction via proton abstraction.
The reaction is possible at either end of GDD molecules. In the process, the benzene ring is covalently bonded to the monomer to become part of the terminal monomer unit of the copolymer. Although initiation could also happen at the unsaturated terminal fatty acid of mcl-PHA, the probability is small owing to low concentration density of mcl-PHA molecule compared to acrylate monomer. Nevertheless, grafting reaction with mcl-PHA does not hinder the propagation of the vinyl polymerization (Fig. 4).

Fig. 4. Initiation and propagation mechanism scheme involving species with alkenyl reactive group $R$ groups that are available within the biogel.
The chain process of the polymerization is continuously repeated to produce a growing macromolecule consisting of a mixture of GDD monomers, acrylate monomers and mcl-PHA with random connection pattern. The cascade of growing copolymer consistently assembles radicalized \( \alpha \)-carbon to be bound to \( \beta \)-carbon of monomers, thereby transferring the radical to the \( \alpha \)-carbon of the newly attached monomer.\(^{25}\) Concurrently, the same process is presumably occurring at the other end of GDD monomers as well. The bivalent nature of GDD, thereby, is giving rise to a cross-linked copolymer. This cross-linking affects the properties of the resulting gel, accounting for almost zero solubility in aqueous solution and a rigid shape. Further complexity of the macromolecule arose from hydroxyl groups in GDD monomer being exposed to dehydration to form carbonyl groups albeit in minute amount due to radical transfer propagation process.

Termination step occurs when two different growing macroradical copolymers are reacting with each other, thereby losing the radical character that is associated with unpaired electrons. Termination from disproportionation is more likely than combination of radicals as shown in Figure 5.

During the grafting process, GDD monomers may also react with each other to form a densely cross-linked homopolymer gel following the same mechanism that applies for the grafting process. Owing to the divalent character of GDD and the nature of the vinyl polymerization, a high molecular weight is achieved. The crosslinking converts most of the monomers into a highly crosslinked gel, which consists of only a few interwoven polymer networks. A separation of these is
practically impossible. However, GDD-polymers with low crosslinking, owing to incorporation of substantial contents of mono-valent acrylates, may be separated from grafted mcl-PHA based on its solubility in methanol, while highly cross-linked gels and polymers containing higher portions of mcl-PHA are expected to form a precipitate.

In general, the grafted material may comprise of mcl-PHA with varying degrees of grafting component based on different reaction parameters, depending on the initiator concentration, temperature and the concentration of GDD monomer itself.

*Thermal properties of PHA-g-GDD copolymer*

Thermal properties of PHA-g-GDD samples were determined using TGA and DSC analyses. From Figure 6, the grafted samples showed changes in terms of thermal degradation behavior.

![Fig. 6. TGA analysis for PHA, PHA-g-GDD and GDD samples for different initiator concentrations. A & B weight percentage curves, and C & D derivative weight percentage curves. A and C represent samples incubated at 70 °C, while B and D represent samples incubated at 90 °C](image-url)
Neat PHA samples were degraded earlier around 260 °C compared to the rest of the samples (Fig. 6A-D). GDD sample was the most stable among all, with higher degradation temperature around 300 °C. The thermal curves of PHA-g-GDD samples from 90 °C incubation temperature were closer to the GDD curve (Fig. 6), indicating that these samples were more stable compared to those incubated at 70 °C (Fig. 6).

The shapes of the curves were closely related due to molecular composition similarity. From DSC analysis, the $T_m$ of grafted samples was in the range of 53.0 – 55.5 °C, which was slightly higher compared to neat mcl-PHA at 52.7 °C, due to the presence of GDD monomer (Table I).

Table I. Thermal properties of PHA and PHA-g-GDD for different initiator concentrations and incubation temperature, initial rate of reaction, graft yield percentages after two hours incubation, water uptake swelling and porosity percentages

<table>
<thead>
<tr>
<th>$T_{incub}$ / °C</th>
<th>Sample</th>
<th>Initial rate of reaction, % min$^{-1}$</th>
<th>Thermal analysis</th>
<th>Graft yield, %</th>
<th>Water uptake (swelling), %</th>
<th>Porosity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$T_m$/ °C</td>
<td>$T_d$/ °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>mcl-PHA</td>
<td>-</td>
<td>52.7</td>
<td>294.4</td>
<td>~ 0</td>
<td>~ 0</td>
</tr>
<tr>
<td></td>
<td>BPO, mol dm$^{-3}$</td>
<td>PHA-g-GDD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>3.0×10$^{-6}$</td>
<td>1.0</td>
<td>55.5</td>
<td>297.9</td>
<td>72 ± 5</td>
<td>2.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5.0×10$^{-6}$</td>
<td>1.8</td>
<td>53.0</td>
<td>305.3</td>
<td>73 ± 11</td>
<td>4.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>10$^{-5}$</td>
<td>2.6</td>
<td>53.7</td>
<td>298.1</td>
<td>77 ± 2</td>
<td>8.2 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>1.5×10$^{-5}$</td>
<td>3.0</td>
<td>54.5</td>
<td>292.8</td>
<td>90 ± 9</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>90</td>
<td>3.0×10$^{-6}$</td>
<td>4.7</td>
<td>53.5</td>
<td>295.6</td>
<td>73 ± 4</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>5.0×10$^{-6}$</td>
<td>4.8</td>
<td>54.7</td>
<td>295.0</td>
<td>86 ± 6</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10$^{-5}$</td>
<td>4.6</td>
<td>53.8</td>
<td>291.6</td>
<td>88 ± 3</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1.5×10$^{-5}$</td>
<td>4.4</td>
<td>53.9</td>
<td>298.7</td>
<td>85 ± 3</td>
<td>9.7 ± 0.5</td>
</tr>
</tbody>
</table>

*Maximum standard deviation ± 10 %

Generally, the thermograms were only slightly different from each other, since the grafting parameters viz. mcl-PHA concentration and GDD concentration were the same throughout the sample preparation reaction. On the other hand, the increase in initiator concentration contributed to the increment in graft yield (Table I), hence resulting in more thermostable functionalized product with higher GDD concentration that were successfully grafted to the mcl-PHA backbone. The findings also agreed with the results from TGA analysis (Fig. 6).

In terms of water absorption ability, the grafted samples exhibited increment in swelling percentage with higher initiator concentration used, although grafted samples from lower incubation temperature showed slightly less water absorption. Neat PHA material showed negligible water absorption due to its strong hydrophobicity. For samples obtained from grafting reaction at 70 °C and different initiator concentrations, approximately similar porosity percentages were determined except at the lowest concentration of BPO used (Table I).
attributed to lower degree of grafting. For grafted samples from 90 °C incubation, the increase in starting radical initiator concentration resulted in lower porosity percentages (Table 1). Neat PHA material showed no evidence of porosity. It is suggested that the increase in initial concentration of BPO and incubation temperature may have contributed to more extensive grafting of PHA that resulted in lower porosity percentages of the resulting materials.

Effects of starting initiator concentration, incubation temperature and time

The grafting reaction of mcl-PHA with GDD made use of BPO as the sole initiator. Based on Figure 7A, at 70 °C incubation, graft yield (%) became higher as the starting BPO concentration was increased. Similar trend was observed in Figure 7B for incubation temperature 90 °C. At 70 °C incubation, the rate of increase in graft yield was gradual for lower starting initiator concentration i.e. 3.0×10⁻⁶ mol dm⁻³ and 5.0×10⁻⁶ mol dm⁻³. When its initial concentration was increased to 10⁻⁵ mol dm⁻³ and 1.5×10⁻⁵ mol dm⁻³, steep increase in graft yield with time was observed (Fig. 7A). On the other hand, when incubation temperature was at 90 °C, similar fast rate of graft yield was observed for all starting initiator concentrations used (Fig. 7B). Nevertheless, for both temperatures, the grafting reaction eventually reached a plateau indicating termination of reaction following exhaustion of grafting sites and/or depletion of radical initiator.

Fig. 7. Graft yield as a function of incubation time, for (A) 70 ° and (B) 90 °C incubation temperature. Initial BPO concentrations at 3.0×10⁻⁶, 5×10⁻⁶, 1.0×10⁻⁵ and 1.5×10⁻⁵ mol dm⁻³ for both temperatures

The grafting reaction of mcl-PHA with GDD made use of BPO as the sole initiator. Based on Figure 7A, at 70 °C incubation, graft yield (%) became higher as the starting BPO concentration was increased. Similar trend was observed in Figure 7B for incubation temperature 90 °C. At 70 °C incubation, the rate of increase in graft yield was gradual for lower starting initiator concentration i.e. 3.0×10⁻⁶ and 5.0×10⁻⁶ mol dm⁻³. When its initial concentration was increased to 10⁻⁵ and 1.5×10⁻⁵ mol dm⁻³, steep increase in graft yield with time was observed (Fig. 7A). On the other hand, when incubation temperature was at 90 °C, similar
fast rate of graft yield was observed for all starting initiator concentrations used (Fig. 7B). Nevertheless, for both temperatures, the grafting reaction eventually reached a plateau indicating termination of reaction following exhaustion of grafting sites and/or depletion of radical initiator.

Effects of initial GDD concentration

The plots in Figure 8 showed the graft yields for different GDD concentrations as a function of time. Initiator reaction is considered a fast one hence it is expected to enter termination phase as the concentrations of the radical and/or reactive sites started to deplete. With higher initial GDD concentration, higher graft yields were also observed. Similar trend was evident for different starting initiator concentrations as shown in Figure 7.

Fig. 8. Graft yield as a function of incubation time for different GDD concentrations at 70 °C incubation. Initial mcl-PHA and BPO concentrations were 50 g L⁻¹ and 10⁻⁵ mol dm⁻³ respectively

Initial rate of reaction

The initial rate of grafting at two different temperatures i.e. 70 °C and 90 °C for different starting initiator concentrations is shown in Table I. At 70 °C, the initial rate was increasing gradually from 1.0 % min⁻¹ to 3.0 % min⁻¹ as BPO concentration was increased, suggesting that the BPO dissociation has yet to reach its maximum level. However, at 90 °C, the initial rate of reaction was almost constant within a narrow range of 4.4 % min⁻¹ to 4.8 % min⁻¹ for all initial BPO concentrations studied (Table I). It is suggested that at this temperature, generation of radical initiator from its parent molecule was relatively faster than at 70 °C, hence the higher initial rate of grafting. This is supported by the fact that half-life of BPO is one hour at 91 °C.

CONCLUSION

The graft copolymerization of mcl-PHA with GDD was successfully carried out using benzoyl peroxide as the sole initiator. Elucidation of its mechanism indicates that both species could be incorporated into the same backbone of mcl-PHA polymer consisting of α-β carbon linkage due to the random nature of
radical polymerization involved. The grafted product yields an amphiphilic copolymer with improved wettability, thus potentially refining its facility for cellular interaction. In addition, grafting of mcl-PHA to yield P(3HO-co-3HHx-co-3HD-co-3HDD)-g-GDD adds to the available repertoire of functional materials.

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ИЗВОД
ПОРОЗНИ АМФИФИЛНИ БИОГЕЛОВИ ДОБИЖЕНИ ЈЕДНОСТАВНИМ БИО-СИНТЕТСКИМ ПОСТУПКОМ

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Реакција калемљења глицерол-1,3-диглицерол-диакрилата (триглицерол-диакрилата) (GDD) на поли(3-хидрокси-алканоат) средње дужине бочних алкил ланца (mcl-PHA) је извршена у раствору ацетона у присуству бензиол-пероксида, као иницијатор. Механизам реакције калемљења на полимерне ланце је детаљно приказан и знатно побољшан у односу на претходно описане у литератури. Слободним радицијалом иницирано калемљење омогућава међусобно повезивање mcl-PHA и GDD молекула преко угљеничне везе, при чему настаје умрежена структура полимера са својствима гела. Термичка својства кополимера са различитим садржајем калемљених грана су анализирана у зависности од концентрације иницијатора и GDD мономера, као и времена извођења реакције полимеризације и температуре. Показано је да су апсорпција воде и порозност синтетисаних гелова знатно повећане у поређењу са полазним полимером, mcl-PHA.

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SUPPLEMENTARY MATERIAL TO
Porous amphiphilic biogel from facile chemo-biosynthetic route

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The observed ¹H NMR spectrum for neat mcl-PHA is shown in Figure S1 matched previously published reports¹-⁵. The signal at 2.5 ppm was assigned to α-position methylene (-CH₂-) group bonded to carbonyl (-C=O) group, whereas the β-position methine (-CH) was found at 5.2 ppm (Fig. S1).

![Fig. S1. ¹H NMR spectrum for neat mcl-PHA](image)
The remaining hydrogen atoms represent the side chain of the hydroxy fatty acid; the methylene protons were found at 1.3 ppm and the terminal methyl group (-CH₃) at 0.9 ppm (Fig. S1).

Another side reaction that leads to additional acrylate monomers, thereby increasing structural complexity of the copolymer, is a (partial) hydrolysis of GDD, as shown in Figure S2.

Fig. S2. Thermal degradation of GDD to produce acrylate monomers via hydrolysis

The terminal species with benzene ring plays a key role as radical to attack an alkenyl group of available GDD monomer, acrylate monomer, or mcl-PHA chain with alkenyl group. The β-carbon of the new species will be covalently bound to the α-carbon of the next (Fig. S4). When a radical attacks the β-carbon of an acrylic system vis-à-vis acrylates like GDD and its derivatives, and olefin-terminated PHA, the resulting radical is resonance-stabilized by the carbonyl group. The delocalization of the unpaired electron, as shown in Figure S3, reduces the energy of the intermediate and is responsible for the regioselective connection of α- to β-carbons⁶.

Fig. S3. BPO dissociation prior to initiation step during grafting commencement

¹³C NMR spectrum for PHA has been reported previously⁷. In this study, solid-state ¹³C NMR analysis spectrum is shown in Figure S5. Since major composition of the biogel are virtually insoluble in any organic solvent, solid-state NMR analysis was initially conducted for PHA-g-GDD copolymer structural analysis (Figure S5).
Fig. S4. The allylic resonance involved after radical intervention on the reaction species. (a) benzene radical attacked the species with alkenyl group, (b) the allylic resonance shows the movement of the unpaired electron along the bonds, and (c) entering propagation step

However, the signal resolution was poor with several combined peaks especially for aliphatic component and the copolymer backbone chain, making it virtually impossible to decipher. The aliphatic component peaks were labeled as A, A’ and A” across 40 ppm to 10 ppm within the spectrum.

Since 1H NMR from the main text allows for structural authentication of low molecular weight and partially soluble grafted product in deuterated chloroform, 13C solid-state NMR further illustrating the complexity of the macromolecule with respect to the insoluble major fraction. Additional signal of minute amount of carbonyl ketone groups are available around 210 ppm on 13C NMR spectrum, labeled as E indicating there was oxidation of secondary alcohol hydroxyl group within GDD, occurring as a side reaction due to the cascading radical reaction environment. However, the distribution of the carbonyl ketone is random throughout the biogel.

A broad combined peak across 80 to 40 ppm represents carbonyl ester carbons, available in both PHA and GDD labeled as B, ether carbons within GDD labeled as B’, and β-carbon within PHA labeled as B”. In addition, initial benzene attachment is clearly visible in 13C NMR spectrum labeled as C between 120 ppm to 110 ppm. On the other hand, a broad peak labeled as D ranging from 170 ppm to 155 ppm is believed to typically from carboxylic groups within PHA and GDD, while peak labeled as D’ is attributed to terminal carboxylic acid group of PHA chain and acrylate component from hydrolysed GDD.
Fig. S5. Solid state $^{13}$C NMR spectrum (bottom) with proposed general molecule of PHA-g-GDD biogel (top)
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