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Poly(3-hydroxybutyrate)-ethyl cellulose based bio-composites with novel characteristics for infection free wound healing application

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ABSTRACT

A series of bio-composites including poly3-hydroxybutyrate [P(3HB)] grafted ethyl cellulose (EC) stated as P(3HB)-EC were successfully synthesised. Furthermore, natural phenols *e.g.*, *p*-4-hydroxybenzoic acid (HBA) and ferulic acid (FA) were grafted onto the newly developed P(3HB)-EC-based bio-composites under laccase-assisted environment without the use of additional initiators or crosslinking agents. The phenol grafted bio-composites were critically evaluated for their antibacterial and biocompatibility features as well as their degradability in soil. In particular, the results of the antibacterial evaluation for the newly developed bio-composites indicated that 20HBA-*g*-P(3HB)-EC and 15FA-*g*-P(3HB)-EC bio-composites exerted strong bactericidal and bacteriostatic activity against Gram$^-$ *E. coli* NTCT 10418 as compared to the Gram$^+$ *B. subtilis* NCTC 3610. This study shows further that at various phenolic concentrations the newly synthesised bio-composites remained cytocompatible with human keratinocyte-like HaCaT skin cells, as 100% cell viability was recorded, *in vitro*. As for the degradation, an increase in the degradation rate was recorded during the soil burial analyses over a period of 42 days. These findings suggest that the reported bio-composites have great potential for use in wound healing; covering the affected skin area which may favour tissue repair over shorter periods.

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1. Introduction

Cellulose is the most abundant renewable material widely used in the history of mankind. In recent years, with the increasing knowledge of infectious diseases caused by various micro-organisms, the development of bio-polymers with multi-functional properties has gained considerable attention, especially in bio-medical and other health-related areas of the modern world. Furthermore, preparations of such structured materials in the form of composites by combining the advantage of various biopolymers like cellulose are among potential routes to impart and/or modify existing properties of natural polymers [1]. Over the last few years, many polymer researchers have directed their interests into the development of structured materials with multi-functional characteristics for wider range of applications like bio-medical, pharmaceutical, drug delivery, antibacterial active packaging and/or sanitary materials, and household items [2].

In our previous work, P(3HB)-EC-based novel composites were developed and analysed the improved thermo-mechanical characteristics and hydrophobic and/or hydrophilic balance obtained [3]. Most phenols, as natural antimicrobial agents, can be grafted onto cellulose surface to impart antimicrobial properties. The cross-linking behaviours of various phenolic molecules including HBA was evidenced after treatment with laccase from *Pycnoporus cinnabarinus* and others [4,5].

Laccase-assisted grafting has recently been the focus of green chemistry technologies in response to the growing environmental concerns, legal restrictions and advances in science. In principle, laccase-assisted grafting may modify and/or impart a variety of new functionalities to the materials of interest, as the modified materials through grafting have extensive applications [6–8]. Oxidoreductases like laccase have considerable potential to react with a large variety of suitable substrates *e.g.*, phenols. This is because their redox potentials are sufficiently low to allow electron transfer by Cu1 reaction site of the laccase. Over the last few decades laccases have been established

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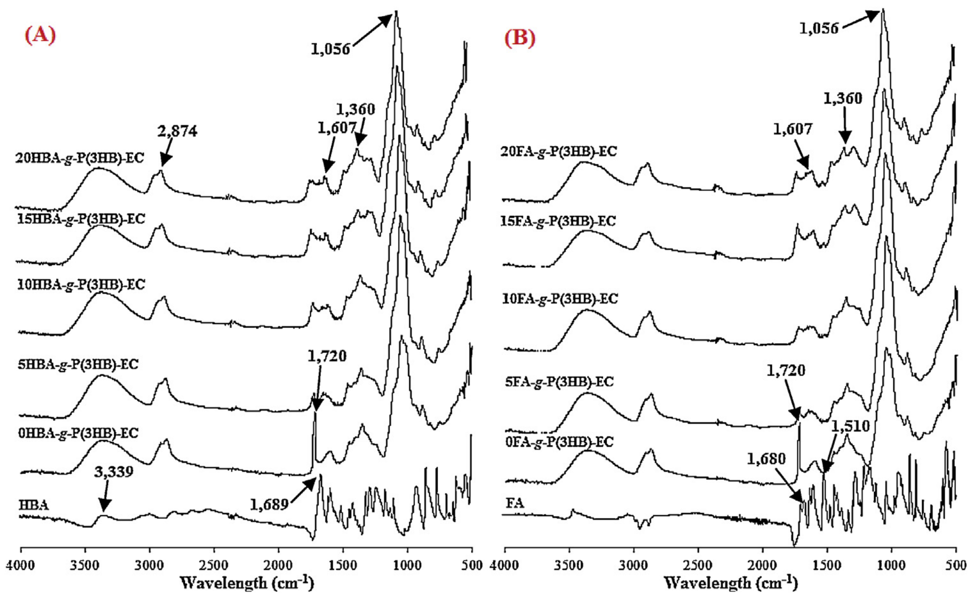

Fig. 1. FT-IR spectra: (A) HBA and HBA-g-P(3HB)-EC bio-composites *i.e.*, 0HBA-g-P(3HB)-EC, 5HBA-g-P(3HB)-EC, 10HBA-g-P(3HB)-EC, 15HBA-g-P(3HB)-EC and 20HBA-g-P(3HB)-EC and (B) FA and FA-g-P(3HB)-EC bio-composites *i.e.*, 0FA-g-P(3HB)-EC, 5FA-g-P(3HB)-EC, 10FA-g-P(3HB)-EC, 15FA-g-P(3HB)-EC and 20FA-g-P(3HB)-EC.

as green catalysts. There is special interest on laccases due to their ability to generate phenoxy radicals as the primary oxidation products. These radicals can further undergo cross-linking reactions [9–11], thus enhancing/modifying characteristics of existing material, and/or imparting new features to the materials, creating value-added products. Laccase-assisted bio-grafting is a versatile method of functionalisation, which allow bonding of various functional groups from diverse phenolic structures [2,12].

The tested phenolic structures have pronounced antibacterial features against various bacterial strains, and exert strong pharmacological activities such as flavouring, antioxidant and antiseptic characteristics, has already been reported elsewhere [13,14]. In continuation of our previous study [3], herein, we report a new study on the development of bio-composites with novel characteristics through enzymatic grafting.

2. Materials and methods

2.1. Chemicals

The enzyme used for grafting purposes was a commercial laccase from *Trametes versicolor* (Sigma-Aldrich, UK). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and natural phenols *i.e.*, *p*-4-hydroxybenzoic acid and ferulic acid were supplied by Sigma-Aldrich, UK. Dulbecco's modified eagle's medium (DMEM), phosphate buffer saline (PBS), streptomycin and penicillin were provided by Lonza, Wilford Industrial Estate, Nottingham UK.

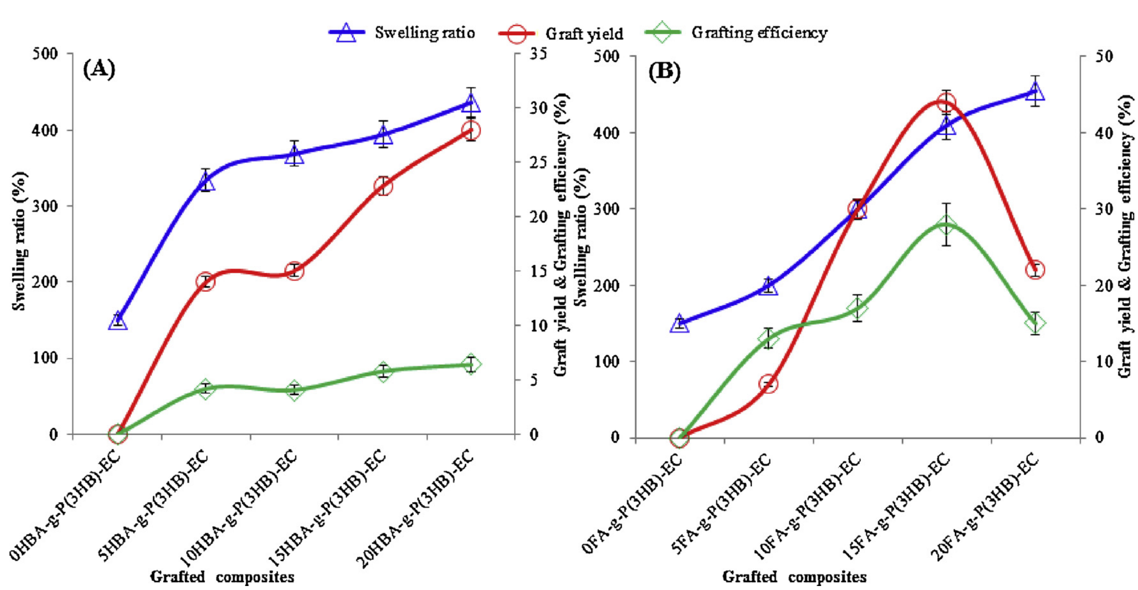

Fig. 2. Graft yield (%GY), grafting efficiency (%GE) and swelling ratio (%SR) behaviours of HBA-g-P(3HB)-EC bio-composites (A) and FA-g-P(3HB)-EC bio-composites (B).

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