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Inherent antibacterial activity and in vitro biocompatibility of hydrophilic polymer film containing chemically anchored sulfadiazine moieties

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ABSTRACT

Microbial colonisation of synthetic materials is a great concern in many fields, e.g., in implant surgery and medical devices; therefore biocompatible hydrophilic organic materials with inherent antimicrobial properties are of current research interest. In this work, we describe the preparation of antibacterial and biocompatible polymeric film based on *N*-vinyl-2-pyrrolidone (VP) and 2-hydroxyethyl acrylate (HEA), using ethyleneglycol dimethacrylate (EGDMA), and synthetic acrylic monomer containing sulfadiazine chemically anchored. The synthesised polyvinylpyrrolidone (PVP)-based films were characterized by different techniques (^{1}H and ^{13}C NMR, ATR-FTIR, SEM, and TGA). In this study, the biophysical responses of bacteria and L929 cells towards the prepared materials as model device surfaces were evaluated. The membrane that contains the anchored sulfadiazine moiety showed excellent antibacterial activity against *Escherichia coli* as well as good biocompatibility. Based on the experimental results, this material is a good candidate for medical applications as a biomaterial.

1. Introduction

The microbial colonisation of synthetic materials is one of the main concerns of several industries such as water treatment, general consumer goods and biomedical implants and devices, to name but a few [1,2]. This is especially concerning when it comes to implant surgery and medical devices where there has to be a balance between antibacterial properties and biocompatibility [3]. Extensive research has been conducted on antibacterial properties of materials [2,4,5]. However the threat of emerging and widespread bacterial resistance to antibiotics [6] which are by far the most commonly used antimicrobial pharmaceutical agents, is endangering their efficacy [7]. In addition to this, new drug development by the pharmaceutical industry has been stalled [8]. Therefore, the lack of new alternatives to treat bacterial infections has become a major global concern that needs to be addressed.

Although “ESKAPE” bacteria are currently studied due to their importance in hospital-acquired infections, *Escherichia coli* is especially prevalent as it is the cause of more Gram-negative infections than *Klebsiella pneumonia* and *Enterobacter* species combined [9]. *E. coli* is one of the most frequently isolated pathogens in different types of medical devices and surgical sites [10] and can induce the growth and virulence in pigmenting anaerobes, and consequently, these also colonise the medical devices [11].

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There is a need to develop new materials that are both an alternative to existing antimicrobial treatments and also address the risk of infection from bacteria outside the "ESKAPE" group such as *E. coli*. Once the etiologic pathogen has been identified and/or antimicrobial susceptibility data are available, every attempt should be made to narrow the antibiotic spectrum. This is a critically important component of antibiotic therapy because it can reduce cost and toxicity and prevent the emergence of antimicrobial resistance in the community [6]. One of the methods that have been implemented is the incorporation of different chemical compounds such as antibiotics to the designed materials [12–14].

In this work, we prepared biocompatible materials based on a copolymer composed of *N*-vinyl-2-pyrrolidone (VP) and 2-hydroxyethyl acrylate (HEA). VP and HEA are broadly applied in cosmetics, foods, adhesives, textiles and specifically as biomaterials [15–17]. In addition to this, sulfadiazine (SDZ) was incorporated into the materials to provide them with antibacterial activity of narrow spectrum. This antibiotic is used in certain therapeutic fields [18] and is on the World Health Organization's list of essential medicines [19].

In order to improve the antibacterial efficiency whilst maintaining the biocompatibility, a method to incorporate the antibiotic has been proposed. A sulfadiazine moiety (acrylic monomer containing sulfadiazine) was synthesised and chemically anchored to the co-polymeric film to avoid the migration and residual toxicity of the antibiotic [4,20,21]. Also, this anchorage of the sulfadiazine co-monomer provides the possibility of sustained release over a prolonged period compared to the native monomer of the drug dispersed in the film [4,22].

In summary, biocompatible films with improved antibacterial activity were prepared and characterized by different techniques. Also, their antibacterial properties and biocompatibility were assessed.

2. Materials and methods

2.1. Materials for the synthesis of monomer and films

All compounds and solvents were commercially available and used as received: *N*-vinyl-2-pyrrolidone (VP) (Aldrich, 99%), 2-hydroxyethyl acrylate (HEA) (Aldrich, 96%), ethylene glycol dimethacrylate (EGDMA) (Aldrich, 98%), methacryloyl chloride (Fluka, 97%), 1-methyl-2-pyrrolidone (NMP) (Aldrich, 99%) and sulfadiazine (SDZ) (4-amino-*N*-(pyrimidin-2-yl)benzenesulfonamide) (Alfa Aesar, 99%). Azo-bis-isobutyronitrile (AIBN) (Aldrich, 99%) was recrystallized twice from methanol.

2.2. Synthesis of sulfadiazine-containing acrylic monomer

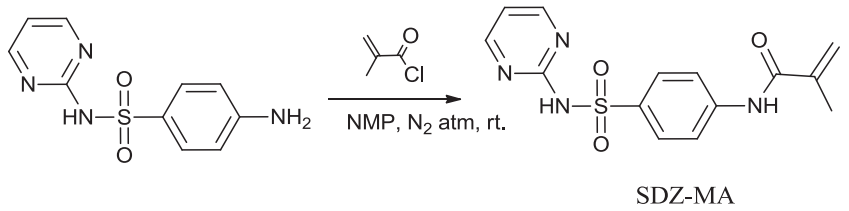
The synthesis of sulfadiazine-containing acrylic monomer *N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)methacrylamide (SDZ-MA) was performed as follows: 5 g (20 mmol) of sulfadiazine (SDZ) was dissolved in 20 mL of NMP in a round-bottom flask under nitrogen. Afterwards, 2.3 mL (24 mmol) of methacryloyl chloride was added dropwise. It was stirred at room temperature for 4 h. The solution was added dropwise to water (500 mL) under vigorous stirring, yielding a white solid product that was obtained after filtering. Then it was washed three times with 15 mL of water and dried under vacuum at 40 °C overnight. Yield: 5.7 g, 90%). ^{1}H-NMR (δ_H ppm) (400 MHz, DMSO-d_6, Me_4Si): 10.17 (s, 1H), 8.49 (d, 4.9 Hz, 2H), 7.98–7.83 (m, 4H), 7.02 (t, 4.9 Hz, 1H), 5.85 (s, 1H), 5.55 (s, 1H), 1.93 (s, 3H). ^{13}C-NMR (δ_C ppm) (100.6 MHz, DMSO-d_6, Me_4Si): 167.35, 158.38, 156.99, 143.16, 140.01, 134.30, 128.67, 120.98, 119.46, 115.83, 18.65. FT-IR (Wavenumbers, cm^{-1}): ν_{N-H}: 3401, $\nu_{C=O}$ (Amide I): 1695. EI-HRMS *m/z*: calcd for $C_{14}H_{14}N_4O_3S$ 318.0787, found 318.0769. The synthesis is shown in Scheme 1.

2.3. Film preparation

The film F_{BLANK} was prepared by radical polymerization of VP and HEA, using ethylene glycol dimethacrylate (EGDMA) as the cross-linking agent, with a feed co-monomer molar ratio of 68.2/22.7/9.1 for VP/HEA/EGDMA, and AIBN (1 wt%) as a thermal radical initiator. Afterwards, the thermally initiated bulk radical polymerization reaction was carried out in a silanized glass mould of 100 μm thickness under an oxygen-free atmosphere at 60 °C overnight.

The film $F_{SDZ\text{-}MA}$, which contains sulfadiazine moieties chemically anchored to the polymer backbone, was prepared by adding 1% (mole) of the co-monomer SDZ-MA (acrylic monomer with sulfadiazine) to the mixture of monomers used to prepare the blank film F_{BLANK}. Thus, the feed co-monomer molar ratio was of 67.5/22.5/0.9/9.1 for VP/HEA/SDZ-MA/EGDMA respectively and AIBN (1 wt %) as a thermal radical initiator. The polymerization was carried out as described for F_{BLANK}.

The film F_{SDZ} was prepared as described for F_{BLANK} by dispersing SDZ in the mixture of monomers VP/HEA/EGDMA (molar ratio



SDZ-MA

Scheme 1. Synthesis of sulfadiazine-containing acrylic monomer *N*-(4-(*N*-(pyrimidin-2-yl)sulfamoyl)phenyl)methacrylamide (SDZ-MA).