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# Incorporation of biguanide compounds into poly(GL)-*b*-poly(GL-*co*-TMC-*co*-CL)-*b*-poly(GL) monofilament surgical sutures

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ABSTRACT

A new biodegradable coating was developed for bioabsorbable monofilament sutures. Specifically, a random copolymer having 35 wt-% and 65 wt-% of lactide and trimethylene carbonate units showed appropriate flexibility, stickiness and degradation rate, as well as capability to produce a complete and uniform coating. Monofilament sutures of polyglycolide-*b*-poly(glycolide-*co*-trimethylene carbonate-*co*-ε-caprolactone)-*b*-polyglycolide were loaded with chlorhexidine (CHX) and poly(hexamethylene biguanide) (PHMB) to explore the possibility to achieve antimicrobial activity without adverse cytotoxic effects. To this end, two processes based on single drug adsorption onto the suture surface and incorporation into the coating copolymer were used and subsequently evaluated. Although the second process could be considered more complex, clear benefits were observed in terms of drug loading efficiency, antimicrobial effect and even lack of cytotoxicity. In general, drugs could be loaded in an amount leading to a clear bacteriostatic effect for both Gram-negative and Gram-positive bacteria without causing significant cytotoxicity. Release profiles of PHMB and CHX were clearly different. Specifically, adsorption of the drug onto the fiber surface which prevented complete release was detected for PHMB. This polymer had advantages derived from its high molecular size, which hindered penetration into cells, thus resulting in lower cytotoxicity. Furthermore, bacterial growth kinetics measurements and bacterial adhesion assays showed greater effectiveness of this polymer.



## 1. Introduction

Adhesion and proliferation of bacteria on the surface of materials are responsible for severe health problems. Microorganisms can survive on appropriate materials for long periods of time, especially in hospital environments, developing biofilms that could be involved in most chronic infections [1,2], hence the current demand of bacteriostatic, antiseptic and bactericidal agents to prevent bacterial survival and biofilm formation [3–5]. Today, 23% of surgical site infections [6] are caused by Gram-positive *Staphylococcus aureus* bacterium. Specifically, its drug-resistant strain becomes highly dangerous [7] since it could lead to patient mortality and high costs for society [8].

Typical bactericidal agents such as triclosan (TCS), chlorhexidine (CHX) and poly(hexamethylene biguanide) (PHMB) [9] have been employed to prevent bacterial infection. However, other natural agents like bacteriophages [10] can be considered, as well as industrial and clinical agents such as silver [11], quaternary ammonium groups [12], hydantoin compounds [13], and tetracycline antibiotics [14].

CHX (1,1′-hexamethylene-bis-5-(4-chlorophenyl)biguanide) (Fig. 1) has a high activity towards microorganisms [15] as a consequence of the presence of secondary amines that can be protonated, and therefore positively charged under normal pH conditions [16]. Thus, CHX affects the stability of bacterial membranes since it can attach to their negatively loaded (anionic) phospholipids. Furthermore, it has been claimed that CHX may display an anti-inflammatory effect on neutrophil toxic products [17]. PHMB is a cationic oligomer having an average of 7–13 biguanide groups spaced by flexible hexamethylene segments (Fig. 1). The high number of biguanide groups lead to a high effectiveness against microorganisms [18], although chemical characterization is difficulted for the high dispersion of oligomer sizes.

Sutures penetrate through the protective skin and can come in contact with microorganisms that grow in subcutaneous tissues such as hair follicles. Microorganisms can therefore attach to the suture surface, allowing biofilm formation and acting as a niche for subsequent infections [19–21]. Moreover, the risk of infection can be increased by an inflammatory response caused by the suture. These problems are very

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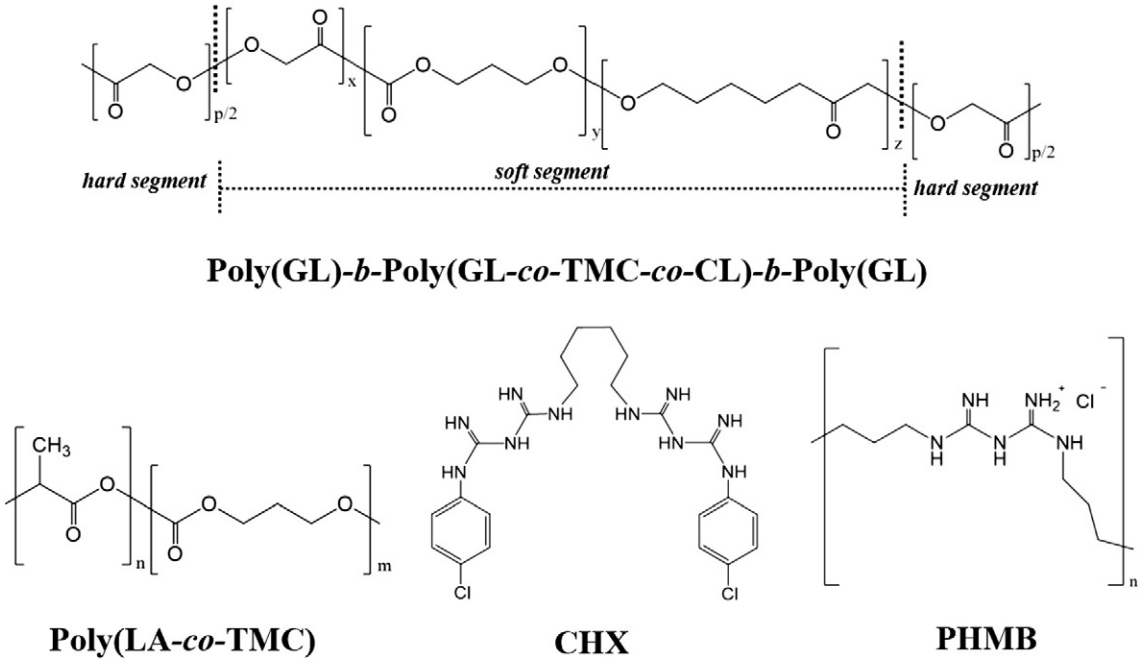


**Fig. 1.** Chemical structures of poly(GL)-*b*-poly(GL-*co*-TMC-*co*-CL)-*b*-poly(GL) (Monosyn™), the coating poly(LA-*co*-TMC) copolymer and the selected CHX and PHMB bactericides.

important for sensitive and risk applications like sutures securing a central venous catheter [21].

Currently, the most commonly used antimicrobial surgical suture is Coated Vicryl Plus Antibacterial Suture, a multifilament suture constituted by a copolymer having 90 wt-% of glycolide and 10 wt-% of L-lactide and TCS deposited on its surface to take profit of its capability to inhibit the colonization of a broad spectrum of bacteria [22]. Nevertheless, the incorporation of other bactericides is strongly recommended for the following reasons: a) The increasing resistance of bacteria to TCS caused by its massive use [23], and b) Safety issues concerning the bioaccumulation of TCS and its negative effect on immune and reproductive functions [24]. In this way, coating formulations based on an amphiphilic polymer, poly[(aminoethyl methacrylate)-*co*-(butyl methacrylate)] (PAMBM), have been proposed due to its higher antimicrobial activity at lower concentrations than that detected for TCS loaded samples [25].

CHX has been considered as alternative to TCS; specifically, coatings based on fatty acids (i.e. chlorhexidine laurate and chlorhexidine palmitate) were evaluated using Vicryl Plus as a reference multifilament suture [26]. High antimicrobial efficacy was demonstrated for up to 5 days while acceptable cytotoxic levels were determined for 11 μg/cm drug content.

The use of coatings is essential for multifilament sutures since they have a lubricant effect and can diminish tissue drag and risk of infection caused by capillarity [27,28]. These problems are not found when monofilament sutures are employed but the use of a coating may be still highly interesting if a drug is incorporated. This is studied in the present work using Monosyn™ (i.e. polyglycolide-*b*-poly(glycolide-*co*-trimethylene carbonate-*co*-ε-caprolactone)-*b*-polyglycolide (Fig. 1) abbreviated as poly(GL)-*b*-poly(GL-*co*-TMC-*co*-CL)-*b*-poly(GL)) [29] as a monofilament suture and CHX and PHMB as examples of bactericidal drugs with low and relatively high molecular weights, respectively. In addition, a new coating constituted by lactide and trimethylene carbonate (Fig. 1) (abbreviated as poly(LA-*co*-TMC)) was developed according to the interest of this kind of copolymers for different biomedical applications [30–33]. Composition was selected to obtain a material with a sticky nature and a low degradation rate.

The goals of the present works involve also the evaluation of which is the best way to load the bactericide drug (i.e. direct deposition onto the suture surface or into a coating copolymer), the evaluation of the most effective biguanide compound (i.e. low or high molecular weight samples) and finally the evaluation of the higher drug load that render a bactericide/bacteriostatic effect without causing cytotoxicity.

## 2. Experimental section

### 2.1. Materials

Lactide, trimethylene carbonate and $Sn(Oct)_2$ were purchased from Sigma-Aldrich. Commercially available sutures of poly(GL)-*b*-poly(GL-*co*-TMC-*co*-CL)-*b*-poly(GL) (Monosyn™, USP 0 and diameter 0.35–0.399 mm) were kindly supplied by B. Braun Surgical, S.A. This triblock copolymer was constituted by 72, 14 and 14 wt-% of glycolide, trimethylene carbonate and ε-caprolactone units, respectively. The material had a middle soft segment that represents the 43 wt-% of the sample. Weight average molecular weight was 90,700 g/mol.

All solvents, chlorhexidine (CHX), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and cell culture labware were purchased from Sigma-Aldrich (Spain). Cosmocil® (poly(hexamethylene biguanide hydrochloride), PHMB) was kindly provided by B. Braun Surgical S.A.

The microbial culture was prepared with reagents and labware from Scharlab (Spain). *Escherichia coli* CECT 101 and *Staphylococcus epidermidis* CECT 245 bacterial strains were obtained from Spanish Collection of Type Culture (Valencia, Spain). African green monkey kidney fibroblast-like (COS-7) and epithelial-like (Vero) cells were purchased from ATCC (USA).

### 2.2. Polymerization

Synthesis of the coating copolymer was carried out in tubes previously silanized with a silanization solution type I (Sigma-Aldrich) to prevent chemical reaction between the monomers and the OH groups contained in the glass. Silanization was performed during 30 min and then tubes were washed three times with anhydrous methanol and dried for 24 h in a preheated oven at 120 °C. Copolymers with different ratios of lactide and trimethylene carbonate were synthesized in order to select the composition with better properties to be used as a coating.