

POLYMERIC SPACERS FOR ANTIMICROBIAL AGENTS

C. Waschinski & J.C. Tiller

Freiburger Materialforschungszentrum (FMF), Albert-Ludwigs-Universität Freiburg, Germany.

INTRODUCTION: There is a great interest in modifying surfaces of common objects to render them antiseptic. In the most common applications, surfaces are impregnated with antimicrobial agents, which are released into the surrounding environment. Another approach is based on grafting macromolecules that repel microorganisms (e.g., poly(ethylene glycol)), onto a surface. Grafting antimicrobial polymers [1], such as *N*-alkylated poly(4-vinylpyridine) results in surfaces that are capable of killing microbes on contact [2]. Although the latter concept is the most effective one, it is not fully understood. One hypothesis is that the antimicrobial functions of the grafted polymer are carried through the thick cell wall of, e.g., bacteria using the polymer backbone as long spacer. In order to verify this proposed mechanism, we decided to immobilize biocidal groups via polymeric spacers of well defined and uniform length. It is expected that a minimal effective length of the polymer chain is required to remain the antimicrobial activity of the immobilized function.

Here, we report on the synthesis of narrowly distributed polymeric spacers based on 2-ethyl-1,3-oxazoline carrying functional groups at both ends with one of these functions being able to attach covalently to a surface and the other group possessing antimicrobial properties.

METHODS: Initiators were synthesized coming from 1-aminohexanol and 1-aminopentanol. The amino-functions were protected with BOC (*tert*-butoxycarbonyl) groups by conversion with BOC carbonate. Subsequently, the hydroxy-groups were transformed into the polymerization initiating sulfonic acid esters by reaction with trifluoromethanesulfonic anhydride and 4-toluenesulfonyl chloride, respectively. The polymerizations of 2-ethyl-1,3-oxazoline using the prepared initiators were carried out in chloroform at 70°C. After 48 h, the reaction was terminated with different primary amines. The introduced secondary amino end groups were converted into potentially antimicrobial functions by permethylation with methyl iodide.

The bacterial susceptibility test of the synthesized spacers was carried out in bacterial suspensions of *Staphylococcus aureus* (gram-positive bacterium) and *Escherichia coli* (gram-negative bacterium).

The determined minimal inhibitory concentration (MIC) is the concentration of the tested polymer at which 99% of the microorganisms are prevented from growing.

RESULTS & DISCUSSION: By using the new BOC-aminoalkyl sulfonic acid esters as initiators 2-

ethyl-1,3-oxazoline was polymerized to give polymers with degrees of polymerization in the range from 20 to 100 exhibiting a narrow molecular weight distribution of 1.11-1.17. Upon termination and subsequent permethylation followed by deprotection of the BOC-protected NH₂ groups of the starting group poly(oxazoline)s with an NH₂ anchor group and a terminal antimicrobial function could be generated. The MICs (Table 1) showed antimicrobial activity comparable to biocides of low molecular weight if the concentrations were related to the actual amount of antimicrobial groups bonded to the one end of the polymer chain. So, polymeric spacers were synthesized with functional groups at one end of the polymer able to attach covalently to a surface and an antimicrobial function at the other end. These polymers can readily be used for surface modifications in order to investigate the mechanism of surface grafted antimicrobial polymers.

Table 1. MICs of a polymer terminated with dodecane bromide followed by quarternaryzation with methane iodide (DP=44, PD=1.11) and the low-molecular biocide dodecyltrimethylammonium iodide.

	<i>S.aureus</i> [μmol/ml]	<i>E.coli</i> [μmol/ml]
Polymer	15	30
Biocide	3-6	3-6

REFERENCES: ¹T.Tashiro (2001) *Macromol Mater Eng* **286**: 63-87. ²J.C. Tiller, C.-J. Chen, K. Lewis, A.M. Klibanov (2001) *Proc Nat Acad Sci U.S.A.* **98**: 5981-85.

ACKNOWLEDGEMENTS: The authors thank the Deutsche Forschungsgemeinschaft for financing this work in the Emmy-Noether-Programm and the Fonds der Chemischen Industrie for financial support.