SYNTHESIS AND CHARACTERIZATION OF DENDRIMER-DRUG NANODEVICES TO TARGET PERIPROSTHETIC INFLAMMATION

Weiping Ren, PhD[2] Admira Bosnjakovic, MS[1,] Tong Shi[2] Manoj K. Mishra, PhD[1,] Rangaramanujam M. Kannan, PhD[1,2]
[1]Department of Chemical Engineering and Materials Science and [2]Department of Biomedical Engineering
Wayne State University as7606@wayne.edu

INTRODUCTION

Nearly 700,000 total joint replacements (hip and knee) are implanted annually in the United States. More than one-quarter of these prostheses show evidence of aseptic loosening, often requiring revisions. The most frequent cause of aseptic loosening is an inflammatory reaction to one or more of the prosthetic components leading to osteolysis, causing loss of supporting osseous tissues, and loosening of the prosthesis. A recent approach to limiting osteolysis has focused on reducing periprosthetic inflammation and enhancing periprosthetic bone quality. Erythromycin (EM), an antibiotic, can be used to treat periprosthetic inflammation, but delivering adequate levels of EM to the site of periprosthetic inflammation, presents a considerable challenge. We developed PAMAM Dendrimer-EM nanodevice to treat periprosthetic inflammation.

MATERIALS AND METHODS

Synthesis of dendrimer conjugates PAMAM G4-OH was reacted to a protected amine linker followed by deprotection to obtain a bifunctional dendrimer. EM was modified to EM-2'-glutarate then conjugated to the bifunctional dendrimer through an amide bond to give Dendrimer-EM conjugate.
Characterization of conjugates Conjugates were characterized by HPLC, ^{1}H NMR, and MALDI-TOF MS. ^{1}H NMR, and MALDI-TOF MS spectra revealed that four molecules of EM were attached to dendrimer.
In vitro release study Conjugate was dissolved in PBS and was stirred continuously in a water bath at 37˚C. At various time intervals, samples were withdrawn and immediately analyzed by HPLC to determine the EM concentrations.
Cytotoxicity, inhibition of NO-production, and bactericidal activity of dendrimer-EM conjugate Murine RAW 264.7 cells were cultured in the presence of free dendrimer, EM and dendrimer-EM conjugate for a defined time period, and then the cytotoxicity (LDH), LPS- induced NO production and bactericidal activity were measured.

RESULTS

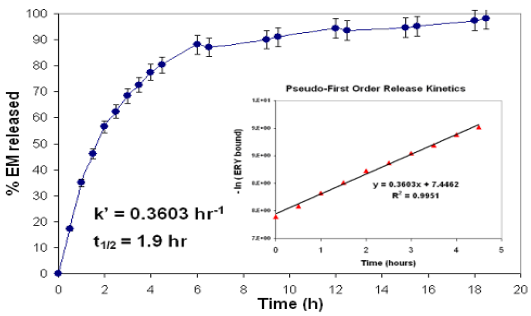
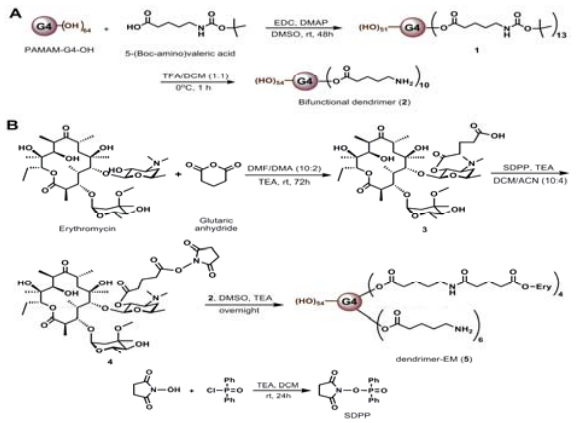


Fig. 2. Drug release profiles of dendrimer-EM conjugates

Fig.1. Synthesis of conjugates. (**A**) Bifunctional PAMAM dendrimer (G4-OH-Link-NH_2, 2). (**B**) PAMAM G4-OH-erythromycin (dendrimer-EM, 5).



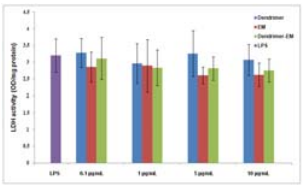


Fig.3. Effect of conjugate on LDH in LPS- stimulated RAW cells

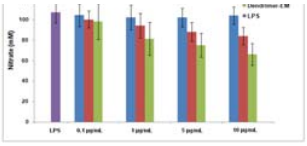


Fig4. Effect of conjugate on LPS-induced NO release in RAW cells.

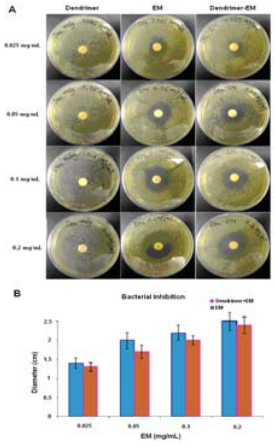


Fig. 5. Zone of inhibition of the conjugate on bacterial growth

CONCLUSION

- PAMAM -EM nanodevice was developed for periprosthetic inflammation treatment.
- Drug payload was ~16% by weight, which is relatively high for polymer conjugates, yet very soluble in PBS.
- Conjugate released the drug effectively, with > 90% of free drug over a period of 10h.
- Conjugate was non cytotoxic to RAW 264.7 cells.
- Conjugate was more effective in reducing NO^- production (a measure of anti-inflammatory activity) compared to free drug, in LPS activated macrophages.
- Antibacterial activity of the conjugate was comparable to free drug.

Abstract #651