Novel Hydrophobic Poly(diol-co-citrates) As Implemented Elastomers For Local Nitric Oxide Delivery

M. C. Serrano & G. A. Ameer.
Biomedical Engineering Department, Northwestern University, Evanston, IL 60201

Statement of Purpose

Advances in the understanding of human cardiovascular pathologies have implicated significant role to nitric oxide (NO) as a signaling molecule with pivotal functions in cardiovascular homeostasis.[1] In the last decades, research has been done to design NO-releasing materials that provide a sustained source of NO for biomedical applications.[2] In our lab, we have recently developed novel amine-containing poly(diol-co-citrates) (PDC) that release nitric oxide in a sustained and localized manner.[3] In this work, we describe the synthesis of novel hydrophobic PDC elastomers as a more efficient polyester-based NO-delivery scaffold.

Materials

Citric acid, 1,8-octanediol, 1,12-dodecanediol, and *N*,*N*-bis(2-hydroxyethyl)-ethylenediamine were purchased from Sigma (St. Louis, MO).

Methods

PDC elastomers containing citric acid (100mmol), 1,12-dodecanediol (90mmol), and *N*,*N*-bis(2-hydroxyethyl)-ethylenediamine (10mmol) were synthesized by condensation reaction (**Figure 1**). The resulting cross-linked elastomers were named as PDDCDA10 and compared to their counterparts containing octanediol (POCDA10). Both polymers were exposed to highly pressurized NO to obtained the diazeniumdiolation of the secondary amine groups as previously described.[4]

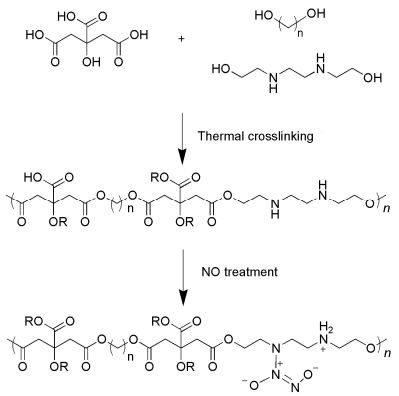

Figure 1. Synthesis of NO-releasing PDC elastomers.

Chemical analysis of the polymer network was then performed by using a FTS40 Fourier transform infrared spectrometer (BioRad Hercules, CA). Tensile mechanical tests were conducted on an Instron 5544 mechanical tester equipped with a 500N load cell (Instron Canton, MA).

NO release experiments were conducted in Phosphate Buffer Saline at 37ºC and indirectly monitored by measuring nitrites in the supernatants through the Griess reaction. Finally, the ability of the amine-containing PDC elastomers to support cell adhesion and proliferation was evaluated with human umbilical vein endothelial cells (HUVEC) and human aortic smooth muscle cells (HASMC).

Results

FTIR spectra from POCDA10 and PDDCDA10 elastomers confirmed the formation of the polyester network. The incorporation of the diaminediol at these concentrations did not significantly affect the elastomeric properties of PDC. Furthermore, both POCDA10 and PDDCDA10 polymers were confirmed to release NO in a sustained and localized manner (**Figure 2**). The burst initial release was significantly decreased when a more hydrophobic diol was used.

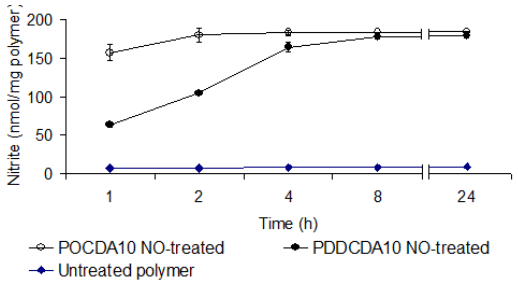

Figure 2. Cumulative release from amine-containing PDC elastomers.

Both POCDA10 and PDDCDA10 elastomers were demonstrated to support adhesion and growth of HUVEC and HASMC (**Figure 3**). Both cell types were able to maintain their characteristic phenotype and form confluent cultures on these materials.

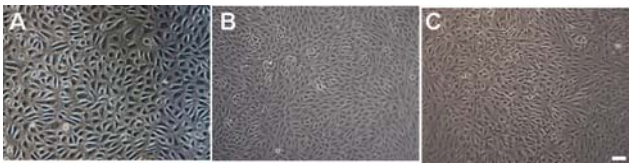

Figure 3. Microscopic images of confluent HUVEC cultures on TCP (A), POCDA10 (B), and PDDCDA10 (C). Scale bar: 25μm.

Conclusions

Novel hydrophobic NO-releasing PDC elastomers have been synthesized. These functionalized polyesters retain elastomeric properties, release NO, and support vascular cell growth. These materials can be considered as promising candidates for NO-based therapies in soft tissue engineering applications. Studies comprising *in vivo* implantation in a vascular injury model are currently underway.

Acknowledgements

M. C. Serrano is indebted to the *Ministerio de Ciencia e Innovación* of Spain for a postdoctoral fellowship.

References

[1] Napoli and Ignarro. Arch Pharm Res. 2009;32:1103-1108.
[2] Varu et al. Vasc Endovasc Surg. 2009;43:121-131.
[3] Zhao H et al. J Biomed Mater Res A. 2009. *In press*.
[4] Hrabie and Keefer. Chem Rev. 2002;102:1135-1154.