A pro-healing PolyHEMA scaffold as an antibiotic-releasing insert for a scleral bandage

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Statement of Purpose: Eye injuries have become one of the most common forms of battlefield injury. It is estimated that 13 percent of wounded Iraq war veterans have sustained direct, penetrating eye damage, as a result of modern weaponry that unleashes an explosive cascade of fragments. Penetrating, combat-related open-globe injuries are associated with intraocular insertion of foreign bodies and it may take up to a week to provide the wounded soldier with an adequate medical assistance. Meantime, the insertion of a foreign body may lead to infection and fibrotic healing that can compromise successful outcomes of treatment and lead to loss of vision. Here we report a fabrication of poly(hydroxyethyl methacrylate) (polyHEMA) based scaffold that releases an antibiotic over the period of one week and is designed to promote non-fibrotic healing. This scaffold can be used as an insert in scleral bandage to stabilize the wound by preventing fibrotic healing and infection until the injured soldier receives professional medical help.

Methods: The scaffolds were fabricated by the sphere tempating tehnique[1,2] and by UV polymerization of HEMA with acrylic acid (AA) and 4-fluorostyrene (FS) at various molar ratios. 10x1 mm dry scaffold disks were soaked in 5 mL of Norfloxacin (NF) in water (0.28 mg/mL), chloroform or ethanol (2.5 mg/ml) and then dried by lyophilization or vacuum. The amount of encapsulated drug was calculated subtracting the dry weight of the scaffold after and before encapsulation. To study the drug release 10mm x 1 mm dry, drug-loaded disk (triplicates) was placed in 3mL of PBS in a temperature controlled shaker incubator set on 37 °C. At each interval of time PBS solution was withdrawn and replaced with 3 ml of fresh PBS. NF concentration was measured spectrophotometrically at 273 nm and determined from the standard curve. A Coupon Evaluation Flow Cell (BioSuriface, Montana, USA) system was used to test the efficacy of drug-loaded scaffolds in comparison to drug-free control scaffolds (triplicates). Scaffolds within the flow cell chamber were inoculated with $2x10^3$ CFU/ml *Staphylococcus epidermidis* (RP62A) and bacterial cells were allowed to adhere for 2 hours. Fresh TSB media was then continuously pumped through the system (1ml/min) for 1, 3 and 7 days. At these time points the scaffolds were removed from the flow cells, rinsed with PBS, sonicated (3 x 30s cycles) to remove bacteria cells, and plated on TSA to allow colony counts to be performed. The survival (%) of the bacteria on drug-loaded scaffolds was calculated relative to the control drug-free scaffolds.

Results: The sphere-templating technique resulted in scaffolds with uniform 38 μm pore diameter based on co-polymers of HEMA with AA and FS at different ratios. Drug encapsulation for all compositions was more efficient in organic solvents compared to encapsulation in aqueous solution. For all drug-loading solvents, the amount of NF encapsulated within the scaffold that includes AA in its composition was higher compared to the amount of NF encapsulated in scaffolds without AA. However, the kinetic of drug release from compositions with AA was significantly faster compared to the co-polymer of HEMA with FS only. For example, poly(HEMA-AA) and pol(HEMA-AA-FS) scaffolds that were loaded with NF in ethanol stop releasing the drug after 3 days, while poly(HEMA-FS) scaffolds release the drug during 2 to 4 weeks, as molar percent of FS increases from 2.5 to 5%, respectively. During a flow cell experiment, the drug-loaded poly(HEMA-FS) (2.5% molar FS) scaffold showed inhibition of 98.8, 95.8 and 71.4% of *S. epidermidis* growth after 1, 3 and 7 days in flow cell, respectively. Figures 1A and 1B show SEM images of the control and the drug-loaded poly(HEMA-FS) scaffold after 7 day in flow cell. The surface of the control (w/o the drug) is completely covered with bacteria that hide its porous morphology while the population of the bacteria on the drug-loaded sample is significantly lower.

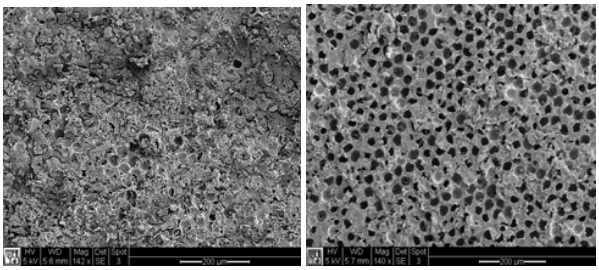

Figure 1. SEM images of poly(HEMA-FS) scaffold surface w/o (the control, A) and with (B) NF, respectively, after 7 days in flow cell.

Conclusions: The optimal scaffold for release of antibiotic is based on hydrogel that is a co-polymer of HEMA and FS. The poly(HEMA-FS) scaffold was successfully loaded with NF and the scaffold releases the drug during over more then one week period in a sustained manner. The scaffold is designed to have pore diameter of 38 μm, the pore size that promotes non-fibrotic healing.[2] The successful encapsulation of the drug within the material and then slow and sustained release are attributed to structural similarity between the NF and FS. The 71.4% inhibition of *S. epidermidis* growth after 7 days in the flow cell is promising since during the experiment in a flow cell the drug is continuously washed away by the flow of a fresh media. The tear flow in the eye is significantly lower comparing to the flow of media in the cell, especially when the eye is under the scleral bandage. Therefore, we expect significantly higher inhibition during an upcoming *in vivo* study. Based on the reported results, the developed poly(HEMA-FS) scaffold could be used as efficient anti-bacterial insert in a scleral bandage.

References: 1) Galperin A. J Biomed Mater Res Part A. 2012; doi: 10.1002/jbm.a.34380; 2) Madden LR. Proc Natl Acad Sci. 2010;107:15211-6.