Evaluation of a Hydrogel-based Antibiotic Release System for Endodontic Therapy

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Statement of Purpose: Over 15 million root canals are performed yearly in the United States[1] to treat pulp inflammation. However, this procedure leads to tooth devitalization and is associated with high incidences of failure[2] and reinfection. Thus there exists an unmet clinical need for endodontic therapy with the potential to restore tooth function, while simultaneously eliminating infection. The **objective of this study** is to design and optimize a hydrogel scaffold for antibiotic delivery and guided pulp regeneration. Specifically, ciprofloxacin will be incorporated into a composite gel of polyethylene glycol and fibrinogen (PEG-F), and the efficacy of antibiotic release for treating anaerobic bacterial infection and potential effects on human dental pulp cell function will be determined. It is anticipated that the PEG-F hydrogel will facilitate ciprofloxacin release, with an optimal antibiotic dose at which bacterial infection is reduced without adverse effects on pulp cells.

Methods: *Ciprofloxacin loaded PEG-F*- PEG-F (10kDa), Irgacure^{TM}2959 (0.3% w/v) were mixed with ciprofloxacin solution and crosslinked under UV (365nm, 10 min). The resultant hydrogel contains 0, 25, or 50 µg/ml antibiotic. *Pulp Cell Response*-Human dental pulp cells were seeded in 24-well plates ($5x10^4$ cells/well), cultured in fully supplemented DMEM with ciprofloxacin (0, 25, 50, 100, 150µg/ml) or PEG-F loaded with ciprofloxacin (0, 25, 50µg/ml). Samples were over 14 days for cell viability (n=2) plus cell proliferation and alkaline phosphatase (ALP) activity (n=6). *Bacteria Culture*-Bacteria from infected human pulp was grown for 20 hours in thioglycollate broth with addition of ciprofloxacin (0, 25, or 50 µg/ml) or ciprofloxacin loaded hydrogels under anaerobic conditions. Turbidities was determined using McFarland standards (n=3) for bacteria growth. *Statistical Analysis* - ANOVA and the Tukey-Kramer *post-hoc* test were used for all pair-wise comparisons ($p<0.05$*over time, # between groups).

Results and Discussion: *Pulp Cell Response*- A dose-dependent decrease in pulp cell number were seen when ciprofloxacin was directly added to the media (Fig 1). Moreover, cell number decreased significantly over time at 100 and 150 µg/ml ciprofloxacin in media ($p<0.05$). In contrast, when ciprofloxacin was released from the PEG-F hydrogel (Fig. 2), no adverse effect on cell number was observed between groups, and cell number increased over time. A significant increase in ALP was seen with 25 & 50 µg/ml ciprofloxacin in PEF-F (Fig 2). *Treating Bacterial Infection*- Effects of both media ciprofloxacin dose and antibiotic released from PEG-F were tested on anaerobic bacteria isolated from three endodontic patients. While differences in bacteria growth was seen between patients, both media addition (Fig. 3) and controlled release of ciprofloxacin (Fig. 4) significantly reduced bacterial turbidity, with no apparent difference observed between 25 and 50 µg/ml groups (2/3 patients). Accumulated hydrogel ciprofloxacin release in bacteria broth after 20-hr incubation was 15.62±0.43 µg/ml for the 25 µg/ml-PEG-F group, and 34.65±1.07 µg/ml for the 50 µg/ml-PEG-F group. Interestingly, % bacteria kill was significantly higher in the 50 µg-PEG-F group for two out of the three patients.

Conclusion: Controlled release of ciprofloxacin from PEG-F was effective in reducing anaerobic infection, without adverse effects on dental pulp cell growth and differentiation. Future studies will evaluate this promising antibiotic delivery system for guided pulp regeneration in vivo.

Reference: 1. ADA. Survey of Dental Practice. Chicago: ADA; 2007 2.Friedman S. Oxford: Blackwell Science. 1998: 367-401

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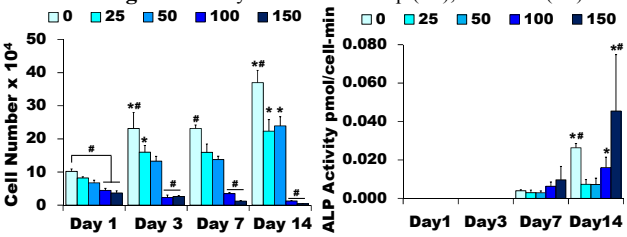

Figure 1. (A) Cell number and (B) ALP activity over time of pulp cells cultured in media supplemented with different ciprofloxacin concentration (µg/ml, *, # $p<0.05$).

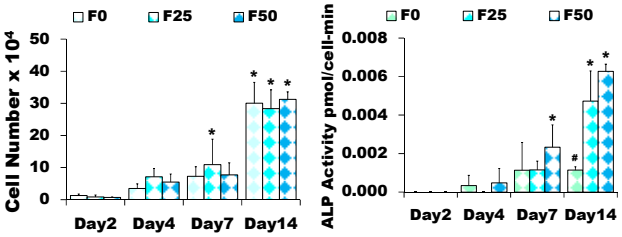

Figure 2. (A) Cell number and (B) ALP activity over time of pulp cells cultured with ciprofloxacin-loaded in PEG-F hydrogel (0, 25 or 50 µg/ml ciprofloxacin, *, # $p<0.05$).

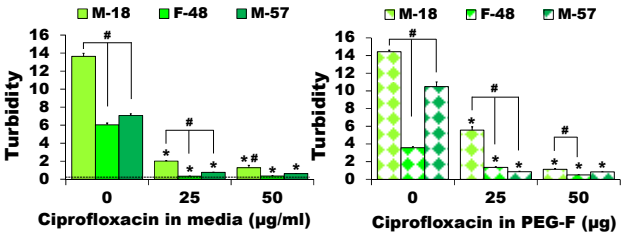

Figure 3. Turbidity bacteria growth in broth with (A) different ciprofloxacin concentration (B) PEG-F with different dosage of ciprofloxacin (*, # $p<0.05$, Male 18&57 yr-old, Female-48 yrs).

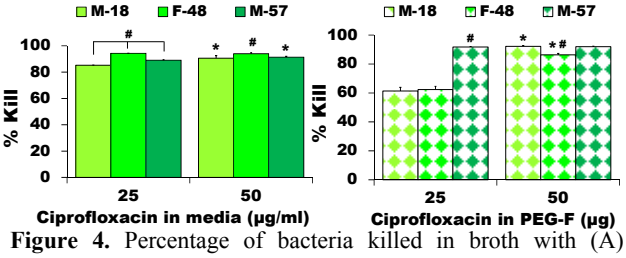

Figure 4. Percentage of bacteria killed in broth with (A) different media ciprofloxacin concentration (B) PEG-F with different dosage of ciprofloxacin (*, # $p<0.05$).