Characterization of Novel Injectable Click-Hydrogel Scaffold for the Treatment of Re-Synostosis

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Statement of Purpose: Craniosynostosis is the pathologic fusion of one or more cranial sutures early in development. Craniosynostosis can lead to severe craniofacial deformities, blindness, deafness, and developmental delays. The standard of care for the treatment of craniosynostosis is the surgical removal of the fused suture(s) in an attempt to restore normal cranial growth. In up to 40% of surgeries the bones of the skull fuse again, resulting in re-synostosis. This re-synostosis necessitates a second surgery to remove the fused bone which is associated with a 15% incidence of life threatening complications such as: dura tears, meningitis, and intracranial hemorrhages. Despite severity of the complications there is no therapy clinically available. The objective of this study was to develop and characterize a novel injectable hydrogel scaffold for the treatment of re-synostosis.

Methods: The hydrogel cross links spontaneously by a cyclo-addition click reaction between a polyethylene-glycol (PEG) monomer containing azide functional groups and our newly developed cross linking molecule. The cross linker contains an octyne ring attached to two benzene rings incorporated to increase the ring strain and decrease the energy of activation needed for polymerization. This allows for *in situ* polymerization without the use of chemical initiators or the production of free radicals.

In-vitro release kinetics and degradation were performed by incorporating 14 ug/mL of Cy3 labeled Glutathione-S-Transferase into 63 mg/mL of the crosslinker reconstituted in PBS. This was reacted in a 2:1 ratio with 0.25 mg/mL s of the PEG-N_3 backbone. The samples were incubated at in the dark at 37 ºC in sterile PBS with 10% FBS for: 3 hours, 1 day, 3 days, 7 days, 10 days, and 14 days. The protein release was quantified by measuring the fluorescence of the solution at the specified time points. Degradation was assessed in separate samples with unconfined compression testing, determination of mass loss, and calculation of swelling ratios.

The in-vivo performance was assessed in our mouse model of re-synostosis. Briefly a 1.5 by 2.5 mm defect was created to remove the posterior frontal suture. The defect was either left empty as a control or 1 uL of the hydrogel was injected and allowed to polymerize *in situ*. The mice were imaged with micro-computed tomography(μCT) 5 and 10 days following surgery. The re-closure of bone in the defect was quantified with our previously developed and validated snake algorithm. To directly image the hydrogel, mice were imaged with a 9.4 T MRI and protein incorporation into the gel was assessed with in-vivo fluorescence imaging.

The data were analyzed by one-way ANOVA and where appropriate statistical significance was determined using the Bonferoni multiple comparison test with $p<0.05$ being considered significant.

Results: Analysis of the linear region on the stress-strain curves from the compression testing showed that at 3 hours the samples had an elastic modulus of 20 kPa. The modulus remained constant until day 5 and then decreased down to 11 kPa by day 14. The release of the fluorescently labeled GST showed 10% release of the protein at the 3 hours time point, a linear increase up to 35% release at 3 days, up release to 90% release of the protein at 14 days (Fig 1A).

The in-vivo MRI imaging showed that the hydrogel was able to polymerize in the defect without disrupting the underlying dura (Fig 1B). The *in situ* fluorescence imaging showed the gel polymerized in the defect with a strong fluorescent signal. The results from our snake algorithm showed that at both 5 and 10 days there were no differences in mass of bone in the defect, distance between the bones, area of the defect, or volume of the defect.

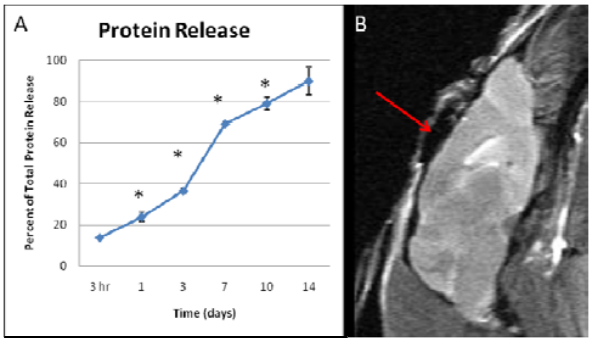

Figure 1. (A) Protein release from hydrogel. (B) MRI showing hydrogel (red arrow) in defect and intact dura. (*=$p<0.05$ vs. previous time point)

Conclusions: The click hydrogel developed shows excellent promise as a scaffold for re-synostosis. The hydrogel showed controlled release of proteins and maintenaned of approximately 50% of the elastic modulus over the time frame studied. Additionally, the increase in the rate of protein release corresponded to the degradation in elastic modulus. For our application the ability to inject the hydrogel into the defect, have it polymerize rapidly, and not damage the underlying soft tissues is of great importance.

The in-vivo data suggest great potential for the hydrogel. The hydrogel scaffold did not impair bone healing at either time point studied. This is ideal for our application because the goal is to delay bone growth, but not prevent it, with the incorporation of bioactive molecules. Current work is being performed to alter the mechanical behavior, degradation, and release kinetics of the hydrogel. Future studies will use the hydrogel as a delivery scaffold for the release of incorporated proteins to delay re-synostosis.

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