Gelatin microparticle-incorporating poly(methyl methacrylate) constructs for craniofacial tissue engineering

Meng Shi,[1] James D. Kretlow,[1] Simon Young,[1,2] Mark E. Wong,[2] F. Kurtis Kasper,[1] Antonios G. Mikos[1,*]
[1]Department of Bioengineering, Rice University, Houston, TX USA
[2]Department of Oral and maxillofacial surgery, University of Texas Health Science Center at Houston, Houston, TX USA

Statement of Purpose: Craniofacial trauma is among the most debilitating forms of injury facing civilian and military populations due to the important aesthetic and functional role of the craniofacial complex. Blast injuries and injuries from high velocity projectiles (e.g., those encountered on the battlefield) often require a staged repair where the surgical revision, however, is sometimes complicated by distortion of surgical landmarks, diminished volume of the defect space, fibrosis of the tissue bed and/or local contamination. Over the course of a staged reconstruction, the placement of a temporary, alloplastic space maintainer may eliminate many of the aforementioned complications. Our laboratory is developing a novel regenerative medicine approach consisting of a first stage using temporary space maintenance to not only maintain the void space but also to prime the wound site for later definitive reconstruction using a regenerative medicine approach. The purpose of this study is to develop a functionalized porous poly(methyl methacrylate) (PMMA) construct as a space maintainer. A local antibiotic drug delivery system, gelatin microparticles loaded with colistin (a polymyxin antibiotic which was chosen specifically to address infections with *Acinetobacter* species, the most common pathogen associated with combat-related traumatic craniofacial injuries), was incorporated into the PMMA construct for treating/preventing local infections, potentially eliminating the infection-related complications associated with space maintenance.

Methods: Microparticles of size less than 250 μm were fabricated from gelatin (isoelectric point 9, Nitta Gelatin Co., Osaka, Japan) and crosslinked with 10 mM glutaraldehyde. Colistin was loaded into gelatin microparticles at a 15 wt% drug loading by co-incubating gelatin microparticles and drug solution for 24 h at 4ºC and then lyophilizing the microparticles. Gelatin microparticle-incorporating PMMA constructs were fabricated by mixing a clinical grade bone cement formulation (SmartSet®, High Viscosity, DePuy Orthopaedics Inc., Warsaw, IN) of PMMA powder and MMA liquid with a carboxymethylcellulose (CMC) hydrogel to induce the surface/bulk porosity and incorporate colistin-loaded gelatin microspheres for drug release. Two formulations of PMMA/CMC/gelatin constructs with 50 wt% CMC and 10-15 wt% gelatin microparticle incorporation were investigated for surface and bulk morphology, porosity, pore interconnectivity by scanning electron microscopy (SEM) and microcomputed tomography (microCT). *In vitro* drug release kinetics were also examined over a period of 5 weeks.

Results and Discussion: The creation of a porous structure in a space maintainer is essential for successful space maintenance because a porous structure allows fibrovascular and other soft tissues to grow into the pores, promoting wound/tissue healing and the formation of a stable interface to anchor the implant in the host tissue. In the PLGA/CMC/gelatin construct, aqueous CMC hydrogel formed micro-coalescences and created a filamentous network throughout the curing material. Resorption of the CMC inclusions in an aqueous environment left voids forming the pore structure (Figure 1A). The bulk porosity was 36±3% for the 10%gelatin-50%CMC construct and 15±2% for the 15%gelatin-50%CMC construct after 1 day dissolution in an aqueous solution, indicating the capability of gelatin and CMC incorporation to induce/tailor the porosity of the PMMA/CMC/gelatin constructs. The *in vitro* release study demonstrated a continuous colistin release over 15 days with a total drug release 63±10% for the construct with higher gelatin incorporation (15 wt%). Relative to the rapid drug elution from gelatin microparticles alone within the initial 2 days, incorporating sufficient amount of gelatin particles in porous PMMA constructs achieved controlled drug release over a prolonged period.

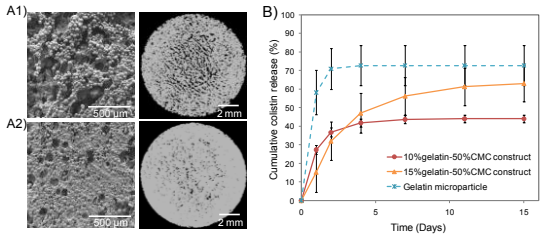

Figure 1. *In vitro* characterization of PMMA/CMC/gelatin constructs: A) surface morphologies of 10%gelatin-50%CMC constructs (A1) and 15%gelatin-50%CMC construct (A2) characterized by SEM (left) on the surface and microCT (right) by the cross-sections after 1 day dissolution in an aqueous solution; B) cumulative colistin release from gelatin microparticles or PMMA/CMC/gelatin constructs over 15 days.

Conclusions: A PMMA/CMC/gelatin composite construct with a porous structure for tissue ingrowth and a controlled drug delivery system for treating/preventing local infections, was created towards application as a temporary implant for space maintenance/infection control during the initial step of a novel two-stage regenerative medicine approach. This study provides insights on the composition parameters that enable viable porosity characteristics/drug release kinetics of the PMMA/CMC/gelatin construct. Future investigation will focus on the evaluation of these PMMA/CMC/gelatin constructs in an infected craniofacial defect model for the role of surface/bulk porosity on the space maintenance capability and controlled drug delivery in treating local infections.

Acknowledgements: This work was supported by a grant from the Armed Forces Institute of Regenerative Medicine (W81XWH-08-2-0032).