Injectable porous biorthogonal click chemistry cement for bone tissue engineering

Xifeng Liu and Lichun Lu
Department of Physiology and Biomedical Engineering and Department of Orthopedic Surgery, Mayo Clinic, Rochester, MN 55905, USA.

Statement of Purpose: Injectable hydrogels and cements are promising candidates for biomedical applications. Current systems often demand free radicals or metal related initiators and/or catalysts for the crosslink process, which may cause severe toxicity to the human body. In addition, the crosslinked dense scaffolds would not allow the cells to infiltrate and form new tissue. Therefore, in this study, we developed an injectable porous "click" organic-inorganic nanohybrids (PO-click-ON) cement that can self-crosslink via metal-free biorthogonal click chemistry and form porous structures via salt leaching. Strain-promoted click reaction enables a fast in-situ crosslink of polymer chains with the exclusion of any toxic initiator and catalyst.

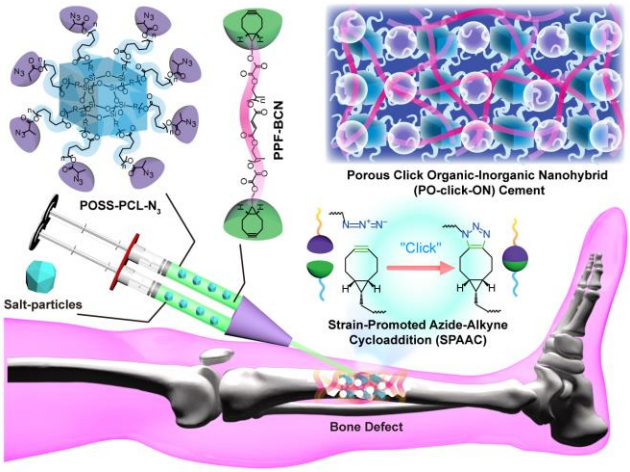

Figure 1. Bone injuries treatment by injectable porous click-ON cement with salt particles. After injection, an osteoconductive cement is formed via crosslinking of the two polymers by catalyst-free strain-promoted alkyne-azide cycloaddition (SPAAC) "click" reaction.

Methods: The injectable system was made by two compositions: poly(propylene fumarate) polymer with cyclooctyne rings (PPF-BCN); and 8-arm polyhedral oligomeric silsesquioxane core with poly(ε-caprolactone) polymers terminated with azide (POSS-PCL-N_3).[1-2] After mixing together, a quick SPAAC click reaction happens to allow *in-situ* crosslinking of the injectable system with the exclusion of any toxic initiator or catalyst (Fig. 1). Salt microparticles were added to form porous structures within the cement by salt leaching. The rat bone marrow mesenchymal stem cells (rBMSCs) were used for *in vitro* cell proliferation and immunofluorescence study. Animal work was proceeded in compliance with the Institutional Animal Care and Use Committee (IACUC) of Mayo Clinic, USA. Sprague Dawley rat critical-sized cranial defect model was used for evaluation of injectability and *in vivo* bone formation of the PO-click-ON cement.

Results: The formation of porous structures within the scaffold by the salt leaching process was successfully achieved (Fig. 2b-c). The resulting PO-click-ON implants supported exceptional stem cell adhesion and osteogenesis on the surface (Fig. 2d-e). *In vivo* study using the rat cranial defect model demonstrated that the PO-click-ON cement achieved outstanding osteogenesis, neovascularization, and new bone formation (Fig. 2f). Histological analysis showed excellent bone formation on the surface and interior of the porous cement (Fig. 2g-j).

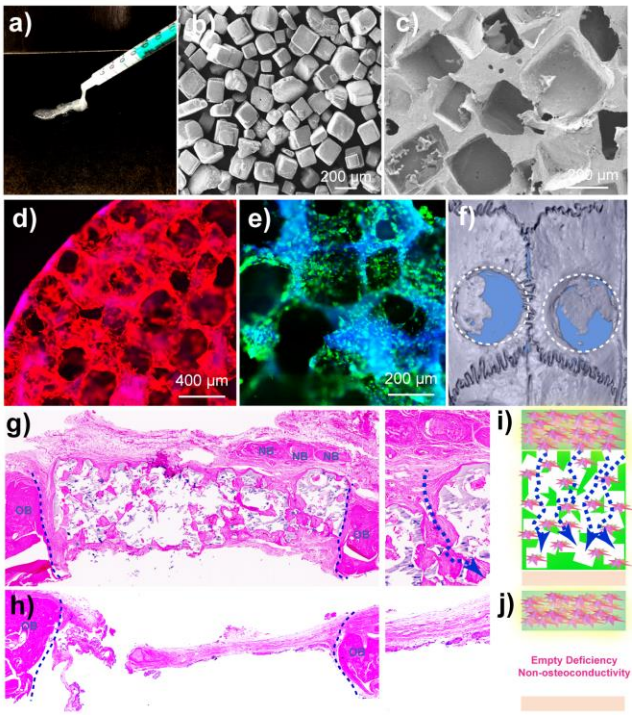

Figure 2. a) Photograph of salt particle incorporated click-ON bone cement. The SEM images of b) salt particles and c) the porous cement after salt leaching. d) The stem cell distribution on the PO-click-ON scaffolds as visualized by F-actin (rhodamine-phalloidin: red) and nuclei staining (DAPI: blue). e) Immunofluorescence staining of osteogenic markers Runx2 protein in stem cells growing on the cement. f) Micro-CT visualization of new bone regeneration in the rat calvarial defect with PO-click-ON cements. H & E staining of tissue slices from the rat calvarial defect sites g) with PO-click-ON cement and h) empty control. Schematic demonstration of i) tissue infiltration within the porous cement and j) the lack of osteoconductivity in the empty defect site.

Conclusions: We report an injectable porous "click" PO-click-ON cement that crosslinks by biorthogonal click chemistry and forms porous structures by salt leaching. The obtained cement showed good stem cell adhesion, proliferation, and osteogenesis. The *in vivo* animal studies demonstrated good bone regeneration. These results suggest that the click cement developed in this study is promising for bone tissue engineering applications.

References: 1. Liu X. Biomaterials, 2021, 276, 121014.
2. Liu X. Biomacromolecules, 2019, 20 (9), 3352-3365.