Injectable bioactive click chemistry polymer cement incorporating BMP-2 and VEGF for spinal fusion in a rabbit model

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INTRODUCTION: Degenerative spinal conditions are common and often necessitate spinal fusion to alleviate pain and restore stability. Current fusion methods, relying on autografts and other materials, have limitations. Injectable polymer systems, recognized for their versatility and applicability in minimally invasive settings, have emerged as promising alternatives. However, traditional injectable systems require toxic initiators or catalysts. Catalyst-free click chemistry, specifically strain-promoted alkyne-azide cycloaddition (SPAAC), offers a solution, enables self-crosslinking without external energy sources or toxic adjuncts. We introduce a novel injectable polymer cement for spinal fusion that employs bioorthogonal click chemistry, combining poly (propylene fumarate)-Bicyclo[6.1.0] non-4-yn-9-ylmethanol (PPF-BCN) and polyhedral oligomeric silsesquioxane-poly(ε-caprolactone) (POSS-PCL-N3) components to achieve rapid crosslinking without toxic agents (**Fig. 1a**). Nano-hydroxyapatite (nHA) and recombinant human bone morphogenetic protein-2 (rhBMP-2) with recombinant human vascular endothelial growth factor (rhVEGF)-loaded microspheres enhance osteogenic and tissue integration properties (**Fig. 1b**). This study evaluates mechanical properties, growth factor release, in vitro osteogenesis, vascular induction, and in vivo bone regeneration in a rabbit spinal fusion model (**Fig. 1c**).

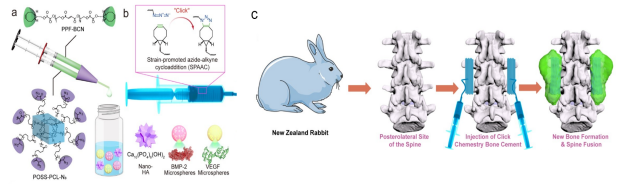

Figure 1: Fabrication of injectable cement and crosslinking of polymer systems via biorthogonal click chemistry.

METHODS: We procured chemicals and materials from reputable sources, including BCN, propylene glycol, Sn(Oct)2, and diethyl fumarate. Synthesis of key polymers, POSS-PCL-N3, and PPF-BCN, followed established procedures. Microspheres, a crucial component, were fabricated using oligo(polyethylene glycol) fumarate (OPF) polymer and loaded with BMP-2 and VEGF growth factors. The injectable cement was prepared by dissolving polymers in N-Methyl-2-pyrrolidone (NMP) solvent, followed by mixing with microspheres and nHA. In vitro experiments involved cell proliferation and differentiation assays. In vivo assessment employed a rabbit posterolateral spinal fusion model, with spine fusion evaluated using imaging techniques. Immunohistochemistry staining was conducted for bone formation analysis. Statistical significance was determined using one-way ANOVA with Tukey post-test as needed.

RESULTS: Microspheres, characterized by spherical morphology and micron-scale size, were successfully incorporated into an injectable cement, serving as effective porogens. Compressive testing revealed a substantial compressive modulus, averaging approximately 1500 kPa (**Fig. 2a**). Growth factor release kinetics demonstrated an initial robust release of rhBMP-2 and rhVEGF growth factors, with rhBMP-2 exhibiting strong release within the first 5 days and both factors showing continuous but slower release over the 24-day study period (**Fig. 2b-c**). The cement exhibited excellent biocompatibility, supporting cell viability and proliferation of rBMSC stem cells and HUVECs similar to controls (**Fig. 2d-f**). Immunofluorescent staining revealed significantly increased osteogenic and vascular differentiation of these cells when exposed to growth factor-loaded cement (**Fig. g-h**). In a rabbit lumbar spinal fusion model, the cement facilitated bone formation, as observed through X-ray and micro-CT imaging (**Fig. 3**), with a dense bone mass bridging transverse processes and facet joints. Histological analysis confirmed neo-bone development, with a polymer cement matrix integrated within, highlighting the cement's remarkable potential for osteointegration and spine fusion applications.

DISCUSSION: The development of an injectable polymer cement for spinal fusion using bioorthogonal click chemistry represents a significant advancement in the field of spine surgery. This novel cement addresses several critical challenges associated with traditional spinal fusion methods, particularly the reliance on autografts and the use of toxic initiators or catalysts in injectable systems. One of the key advantages of the proposed cement is its injectability, making it suitable for minimally invasive spinal fusion procedures. This could potentially lead to reduced surgical trauma, shorter hospital stays, and quicker patient recovery, all of which are highly desirable clinical outcomes. Moreover, the excellent biocompatibility of the cement, as demonstrated in vitro and in vivo, suggests that it may be well-tolerated in the human body, reducing the risk of adverse reactions commonly encountered with other graft materials. The incorporation of growth factors, specifically rhBMP-2 and rhVEGF, into the cement is a pivotal feature. This innovation has the potential to expedite osteogenic differentiation of stem cells and enhance vascularization, crucial factors in achieving successful and rapid spine fusion. While the in vitro findings are promising, further studies, including long term in vivo trials and clinical investigations, are essential to assess the actual impact of growth factor-loaded cement on patient outcomes.

SIGNIFICANCE: The cement's injectable nature holds promise for minimally invasive spinal fusion procedures, potentially reducing surgical invasiveness, postoperative discomfort, and patient recovery times. Moreover, the study's findings emphasize the excellent biocompatibility of the cement, which could mitigate common adverse reactions associated with other graft materials. Notably, the incorporation of growth factors, specifically rhBMP-2 and rhVEGF, into the cement demonstrates its potential to accelerate osteogenic differentiation of stem cells and enhance vascularization, potentially resulting in more effective and expedited spine fusion. Beyond these advantages, the cement offers the potential to address limitations associated with autografts, such as donor site morbidity and limited supply, making it a promising alternative. Additionally, the ability to customize and tailor the cement formulation to individual patient needs underscores its clinical adaptability.

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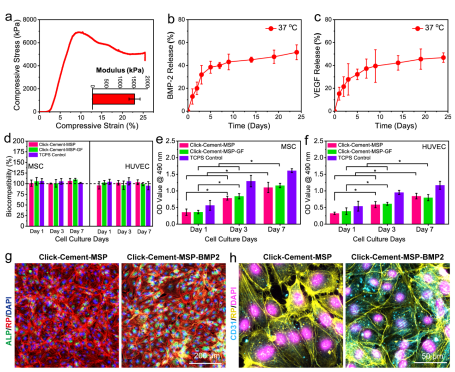

Figure 2: Characterization and in vitro cell studies.

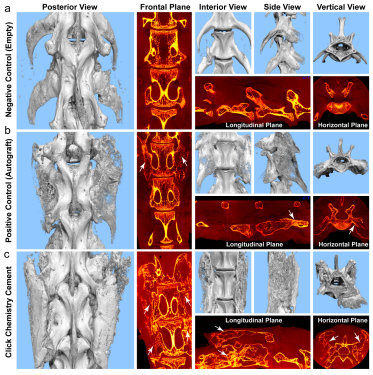

Figure 3: Micro-CT images of the rabbit spine section for a) empty negative control, b) autograft positive control, and c) click chemistry cement visualized from interior, posterior, side, and vertical direction. Arrows indicate new bone