Kartogenin And Bsa-mno$_2$ Nanoparticles Co-delivered Via Hydrogel Promote Chondrogenesis And Cartilage Defect Healing

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Introduction: Focal cartilage defects heal poorly because delivered agents are rapidly diluted and fail to interrupt the inflammatory/apoptotic cascade while stimulating chondrogenesis. We hypothesised that an in-situ-photo-cross-linked F127-DA (Pluronic F127 diacrylate) hydrogel that co-releases bovine-serum-albumin-stabilised MnO_2 nanoparticles (BSA-MnO_2 NPs) and kartogenin (KGN) would localise therapy within the lesion, suppress chondrocyte injury and amplify matrix regeneration.

Methods: BSA-MnO_2 NPs were synthesised and characterised (TEM, DLS, ROS-scavenging, SOD/catalase activity). IL-1β-stimulated human chondrocytes were treated with (i) BSA-MnO_2 NPs, (ii) KGN, or (iii) both delivered from 20 % (w/v) F127-DA precursor that photo-cross-links within 10 s at 37 °C under 405 nm, 50 mW cm^{-2} . NF-κB p65 translocation, cleaved-caspase-3, mitochondrial morphology (MitoTracker, TEM), MMP-13, SOX9, COL2A1 and EdU incorporation were quantified (IF, WB, qPCR). Macrophages were polarised to M1 ± NPs. In 12-week SD rats, 30 μL precursor containing BSA-MnO_2, KGN, or BSA-MnO_2 + KGN was injected into 3-mm trochlear cartilage defects and photo-cured for 10 s (n = 12/group); contralateral knees

received F127-DA-only or no treatment. Repair was evaluated at 4 and 12 weeks by μCT, OARSI histopathology, immunohistochemistry (COL2, MMP-13, cleaved-caspase-3) and mitochondrial ultrastructure.

Results: BSA-MnO_2 NPs abolished ROS, blocked IL-1β-induced NF-κB p65 nuclear translocation and reduced cleaved-caspase-3, while restoring mitochondrial fusion (MFN2) and decreasing fission (DRP1), yielding near-baseline apoptosis . NPs also prevented M1 polarisation. KGN alone elevated SOX9, COL2A1 and proliferation (EdU). Co-delivery from F127-DA hydrogel synergistically maximised anabolism (COL2A1) while preserving anti-inflammatory/apoptotic benefits. In vivo, a single photo-cross-linked F127-DA depot of BSA-MnO_2 + KGN produced the highest OARSI scores at 12 weeks, reduced MMP-13^+ and cleaved-caspase-3, respectively, and restored physiological mitochondrial cristae density versus empty defects.

Discussion: We demonstrate that a single intra-defect injection of a 405 nm-photo-cross-linked F127-DA hydrogel providing sustained co-release of BSA-MnO_2 nanoparticles and kartogenin produces a synergistic, multi-modal repair of critical-size cartilage defects. The NPs act as catalytic ROS sinks that blunt NF-κB nuclear translocation, downstream MMP-13 expression and caspase-3-mediated apoptosis, while simultaneously restoring mitochondrial fusion/fission balance (MFN2↑, DRP1-S616↓). These cytoprotective effects are accompanied by a marked reduction in M1 macrophage polarisation within the defect milieu, suggesting that the early catabolic wave that normally derails endogenous

repair is largely nullified. Concomitantly, KGN drives SOX9-dependent chondrogenic commitment and proliferation, an effect that is amplified when oxidative stress is suppressed by MnO_2. The thermo-responsive F127-DA precursor permits minimally open or arthroscopic injection, conforms to irregular lesion geometry, and cures within 10 s under visible light already used in clinical photopolymerization systems, thereby obviating the need for open fixation or sutures.

Significance/Clinical Relevance: • Minimally invasive single-step procedure compatible with routine arthroscopy (<10 s curing, 405 nm, 50 mW cm^{-2}) • Dual-delivery platform addresses both the “stop catabolism” (ROS/NF-κB/caspase-3) and “start anabolism” (SOX9/COL2) requirements that current cell-free scaffolds or growth-factor-only therapies fail to meet simultaneously • BSA-MnO_2 NPs are biodegradable, FDA-GRAS constituents (Mn^{2+}, albumin), and exert catalytic antioxidant activity at doses ≤50 μg $defect^{-1}$—well below systemic Mn safety margins • KGN is a small, stable, cost-effective molecule amenable to scale-up and off-the-shelf storage; combined intra-articular dose (≈1 μg) is >100-fold lower than systemic exposures in pre-clinical toxicology studies • No exogenous cells or biologics are required, eliminating regulatory hurdles related to viability, sterility and immunogenicity