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A nanopatterned silk-based composite with sustained release of kartogenin for tissue engineering of articular cartilage superficial zone

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INTRODUCTION: Repair of damaged articular cartilage (AC) is very challenging due to its avascularity, acellularity, and restricted regeneration capacity. Despite technology advancement, remodelling of zonal AC has yet to be adequately addressed which underpins proper cartilage biological and mechanical functions. AC is made of four layers with different ultrastructure and function including superficial zone (SZ). SZ makes up 10-20% of the AC thickness, providing smooth joint movement. Since SZ possess the highest tensile strength in AC, its integrity is vital to overcome the mechanical forces from the articulating surface which is challenging when designing a SZ substitute. Here, by considering the silk high strength and chondro-inductive properties of kartogenin as a drug for MSCs chondrogenesis, we aimed to develop a kartogenin-loaded nanopatterned silk platform mimicking the structure and property of AC-SZ.
METHODS: Nanopatterned silk mesh was made by soft and colloidal lithography followed by oxygen plasma etching and N2 plasma ion-implantation technique. Then, kartogenin was immobilized on the silk by 3h incubation with 260 nM drug solution at 37 °C. Finally, oligo-chitosan combined with β-GP was casted on the silk surface and incubated at 37 °C for gelation. In cellular studies, the drug release, biocompatibility, and chondrogenic inductiveness of our platform were evaluated with human adipose derived mesenchymal stem cells (hAMSCs). Experiments were performed in triplicates. Data was analysed by t-test/one-way ANOVA where appropriate with $p<0.05$ considered statistically significant.
RESULTS: The N2 implantation and nanopillar formation on the silk surface reduced water contact angle and improved the adhesion and proliferation of hAMSCs. Kartogenin was continuously released within 30 days linearly, indicating prolonged steady release. The hAMSCs morphology was altered

within 2 days from elongated to nearly ellipsoidal on highly close-packed nanopillars due to small interpillar distance, enabling cells to develop subsequent focal adhesion contacts in all directions. The nanofeature itself upregulated the expression of chondrogenic genes; COL2A1, SOX9, aggrecan and GAG deposition, all of which were further synergized by concurrent KGN release from the silk. The orientation of type-II collagen expressed by hAMSCs on the nanopillars was parallel to the silk surface similar to that of natural SZ. The nanopatterned silk elastic modulus (921.9±46.2 MPa) and the composite shear modulus (10.2 KPa), are close to those of SZ and synovial fluid, respectively. DISCUSSION & CONCLUSIONS: Considering the mechanical performance and the in vitro biocompatibility and chondrogenic differentiation results, this novel structure with prolonged kartogenin release could be considered as an alternative for functional engineering of AC-SZ.

Keywords: Cartilage / joint and arthritic conditions, Drug delivery