Nanoparticle-coated Wavy Scaffolds Enhance Mesenchymal Stem Cell Osteogenesis.

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INTRODUCTION: Large bone defects require therapeutic intervention with grafts for appropriate repair. In addition to osteoinductive chemicals such as BMP2, physical characteristics of the graft are significant factors in long-term success [1]. Surface topography and porosity, for instance, enhances the bone-to-implant contact and mechanical properties of the interface [2]. In addition to the mm-sized topographical features, micro- to nano-meter scaled topographies control the biological response to the implant, where they support induction of osteogenic genes such as osteopontin, BMP2, and RUNX2 [3, 4]. More recently, 3D-printed scaffolds with mm-scale wavy structures was found to enhance osteogenesis [5]. We have developed an electrospun fibrous scaffold with tunable cell-scale wave control [6]. Using water-based synthesis, the fibers are coated with zeolite imidazolate frameworks-8 (ZIF-8) nanoparticles (NP) to introduce surface topography. In this study, we investigate the interactive effects of fiber structure (straight vs wavy), surface topography (via nanoparticle coating), and soluble factors on mesenchymal stem cell (MSC) osteogenesis.

METHODS: Scaffold Fabrication: Polylactic acid (PLA) and poly ethylene oxide (PEO) were electrospun as previously described. The composite was washed to remove the sacrificial PEO and heated to induce fiber wave. For the NP-coated group, materials were immersed in zinc acetate (81.7 mM) and 2-methylimidazole (1 M) solutions for 30 minutes prior to polydopamine-assisted collagen coating [7]. Cell Culture: Human bone marrow-derived MSCs were seeded at 80,000 cells per 1x2 cm scaffold. Control medium consisted of LG-DMEM with 10% FBS, antibiotics, and buffers. Osteogenic medium consisted of control medium supplemented with 0.1 μM dexamethasone, 50 μM L-ascorbic acid-2-phosphate, and 10 μM β-glycerophosphate. Calcium Content: Samples were fixed by paraformaldehyde and stained with alizarin red. 10% cetylpyridinium chloride monohydrate extracted the dye and absorbance was read at 570 nm. Gene Expression: RNA was extracted with the Quick-RNA MiniPrep kit (Zymo Research), and SuperScript IV VILO Master Mix (Thermo) reverse-transcribed the transcripts to cDNA. Expression levels were quantified using the iQ SYBR Green Supermix (BioRad). GAPDH was used as housekeeping gene for control. Immunofluorescence Microscopy: 3-day samples were fixed in paraformaldehyde and probed with DAPI, phalloidin, and anti-vinculin antibody (Sigma). Cell morphology was recorded with a Leica SP5 confocal microscope.

RESULTS: We generated ZIF-8 NPs with a standard hexagonal shape and average diameter of 372 ± 44 nm (Fig 1A). The particles were synthesized in the presence of PLA scaffolds, resulting in NP coating of the fibers (Fig 1B). MSCs seeded on the scaffolds followed fiber structure in all groups, and NP coating enhanced cell elongation and f-actin organization (Fig 2A). Analysis of nuclear shape found significant increases of their aspect ratio in the NP-coated wavy group (Fig 2B), indicating interaction of structure and topography on cell/nuclear morphology. Upon long-term culture with osteogenic medium, MSCs on NP-coated wavy scaffolds expressed increased osteogenic markers, such as alkaline phosphatase (ALP) at day 14 and osteocalcin (OCN) at day 21 (Fig 3A). This finding was confirmed with calcium deposition, where the NP-coated wavy scaffold had the highest calcium content, and a small effect of wavy fiber structure was also observed (Fig 3B).

DISCUSSION: ZIF-8 is a biocompatible NP with applications in bone implant and tissue engineering [8-10]. Combing nano- (ZIF-8-mediated topography) and micro- (fiber structure) scale cues, we find synergistic interactions amongst the topographical, structural, and chemical factors to enhance osteogenic differentiation of MSCs. NP-coated wavy scaffolds result in increased actin cytoskeleton organization and nuclear elongation (Fig 2), which may reflect the increased cell tension, a significant regulator of osteogenesis [11]. The observation is in agreement with our previously report, where wavy cells were found to have enhanced cell traction forces [12]. In addition, nanotopography regulates focal adhesion and receptor co-signaling, demonstrating synergistic action on osteogenesis [13]. An additional mechanism may be the osteoinductive zinc ions released with ZIF-8 degradation [8]. Current studies are investigating the mechanisms behind the synergistic actions of the chemical and physical factors. We are also incorporating osteoinductive reagents, such as dexamethasone or β-glycerophosphate to allow targeted delivery.

SIGNIFICANCE/CLINICAL RELEVANCE: Long-term bone graft success can be improved through optimization of structural and topographical factors. The nanoparticle-enhanced porous fiber scaffold not only can facilitate bone regeneration, it has potential to promote integration with vasculature and soft tissues such as cartilage and ligament/tendon at tissue interfaces.

REFERENCES: [1] Gao X, et al. J R Soc Interface. 2019;16:20190259. [2] Cooper LF. J Prosthet Dent. 2000;84:522. [3] Chen H, et al. Acta Biomater. 2017;59:82. [4] Dalby MJ, et al. Nat Mater. 2007;6:997. [5] Ji S, Guvendiren M. Micromachines (Basel). 2019;11. [6] Szczesny SE, et al. ACS Biomater Sci Eng. 2017;3:2869. [7] Chang C-W, et al. Tissue Eng A. 2020;26:102. [8] Li H, et al. Cellular Reprogramming. 2022;24:80. [9] Hoseinpour V, Shariatinia Z. Tissue Cell. 2021;72:101588. [10] Zhang Y, et al. J Biomater Appl. 2022;36:1042. [11] McBeath R, et al. Dev Cell. 2004;6:483. [12] Chao P, et al. ICBME. 2019. [13] Yang J, et al. ACS Nano. 2014.

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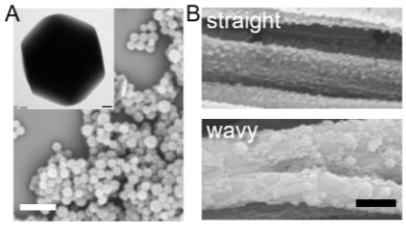

Figure 1. TEM and SEM images ZIF-8 NP. (A) NP alone with white bar = 2 μm in SEM and black bar in TEM insert = 50 nm. (B) NP-coated fibers with bar = 2 μm.

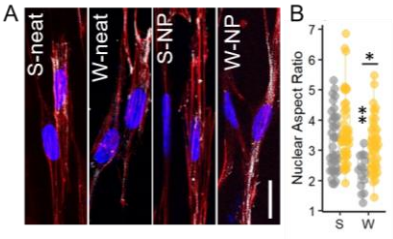

Figure 2. (A) Confocal micrograph of representative MSC morphology (Blue: DNA, red: f-actin, gray: vinculin, bar = 20 μm) (B) Nuclear aspect ratio changes with fiber structure and topography (*p=0.012 vs indicated, **p=0.007 vs S-neat)

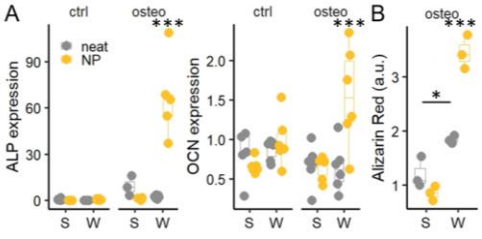

Figure 3. Synergistic interaction of fiber structure, NP coating, and osteogenic factors on gene expression (A) and calcium deposition (B). (***p<0.001 with all other groups, *p=0.03 vs indicated).