Wnt Agonists-Loaded Electrospun Cellulose Acetate Scaffolds For Bone Tissue Engineering Applications

Samir A. Bello, Andrea Vargas-Morales, Joeysel Velázquez-Méndez, and Eduardo Nicolau
Department of Chemistry, University of Puerto Rico at Rio Piedras and Molecular Sciences Research Center, UPR

Statement of Purpose: Bone tissue-engineered constructs are a promising alternative to treat critical-sized bone defects. These constructs are made with porous scaffolds that serve as temporary templates for bone regrowth but generally lack intrinsic cues to attract and activate the progenitor cells *in vivo*. Thus, molecules, mainly growth factors such as BMP-2, have been incorporated in scaffolds to bioactivate them. However, severe drawbacks in clinical trials using BMP-2 loaded scaffolds have prompted the search for alternatives. Recently, there has been a great interest in studying molecules that modulate signaling pathways involved in bone regeneration, such as the canonical Wnt signaling pathway, as potential bioactive factors. No reports of Wnt agonists delivered to bone defects through scaffolds to enhance bone healing have been published. Therefore, here we evaluated the *in vitro* bioactivity of fibrous scaffolds loaded with agonists of the canonical Wnt pathway.

Methods: Firstly, we tested the ability of three Wnt pathway agonists, DIPQUO (Chembridge, San Diego, CA, USA), CHIR99021, and 1-Azakenpaullone (Cayman Chemicals, Ann Arbor, MI, USA), hereafter referred to as DIPQUO, CHIR, and Azak, respectively, to increase the osteoblast differentiation in 2-D cultures. For this, we added DIPQUO (at 5 and 10 μM), CHIR (at 2, 5, 10, and 20 μM), or Azak (at 0.5, 2, 5, and 10 μM) for 12 days to hFOB 1.19 confluent monolayers (ATCC, Manassas, VA, USA) incubated with osteogenic medium. After that, cells were fixed and stained with Alizarin Red, and subsequently, the amount of stain incorporated into the ECM was quantified (Osteogenesis Quantitation Kit, Millipore-Sigma, Darmstadt, Germany). We chose CHIR and Azak for further 3-D studies (in scaffolds) based on their effects on ECM mineralization in 2-D studies. For this, we first solubilized CHIR or Azak in acetone/dimethylacetamide to obtain concentrations of 0.25, 0.5, and 1 mg/mL, and then cellulose acetate (CA) was added to the CHIR or Azak solutions and were stirred ON at 4°C to obtain a final concentration of 17% (w/w) CA in CHIR or Azak containing solutions. Then, the agonist/CA solutions were electrospun to obtain fibrous scaffolds. The micromorphology and surface wettability of electrospun mats were studied by SEM and water contact angle measurements, respectively. The CHIR and Azak release profile from drug-loaded scaffolds was also established. Additionally, the *in vitro* biocompatibility of CHIR and Azak-loaded mats was determined by seeding hFOB 1.19 cells on them for 7 days and counting the number of cells attached on each scaffold along with their morphology and proliferative rate (BrdU incorporation Assay).

Results: Preliminary experiments in 2-D cultures showed that only CHIR and Azak significantly increased the amount of Alizarin Red bound to the mineralized extracellular matrix compared to controls (cells treated with osteogenic medium). Thus, we proceeded to incorporate them separately into electrospun CA nanofibers at three different concentrations (0.25, 0.5, and 1 mg/mL) by blending electrospinning. SEM analyses showed that all the scaffolds (pristine CA scaffolds and those loaded with CHIR or Azak) were composed of randomly oriented nanofibers with diameters ranging from 200 to 800 nm. We found that the incorporation of CHIR or Azak into nanofibers did not alter their micromorphology. Water contact angle measurements indicated that all the scaffolds are hydrophobic (contact angle higher than 90°), but CHIR-loaded mats displayed a reduced hydrophobicity compared to Azak-loaded and pristine CA nanofibers. Remarkably, the release profile of CHIR and Azak from nanofibers to PBS/Tween-80/methanol was different, showing a slower release of CHIR from CA nanofibers compared to Azak. *In vitro* compatibility studies showed that hFOB 1.19 cells were able to adhere on all the scaffolds displaying the normal elongated morphology. However, cell nuclei counting demonstrated that a higher number of osteoblasts were adhered to CA scaffolds loaded with 0.25 and 0.5 mg/mL of CHIR after 7 days in culture compared to the number of cells attached to controls (pristine CA) and Azak-loaded scaffolds. Similarly, the proliferative rate was higher in cells growing in CA scaffolds loaded with 0.5 and 1 mg/mL of CHIR compared to controls (pristine CA) and Azak-loaded scaffolds. Ongoing experiments are evaluating the effect of CHIR and Azak-loaded scaffolds on osteoblast differentiation and ECM mineralization.

Conclusions: Overall, our results demonstrate that the Wnt agonists CHIR and Azak can be successfully incorporated into CA nanofibers by blending electrospinning. However, only CA scaffolds loaded with CHIR (at some of the doses tested) exhibited *in vitro* bioactivity as demonstrated by increased cell adhesion and proliferative rate of cells growing on them. These findings might be related to the reduced hydrophobicity of CHIR-loaded scaffolds and the slower release of CHIR from CA nanofibers compared to Azak.