Novel Fully Biodegradable Biomimetic Scaffolds for Bone Regeneration and Repair

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Statement of Purpose
Restoring lost function to failed or diseased tissues remains a major clinical challenge. Traditional biomaterials and current treatments are often ineffective for tissue regeneration and/or repair. Therefore, there is a great demand for innovative biomaterials to solve these problems. Highly bioactive and readily available tissue substitutes would revolutionize the current treatments for bone tissue loss and failure due to trauma, disease, or congenital defects, as well as significantly reduce annual health care costs. Two of the most critical factors of a scaffold in promoting tissue regeneration and/or repair are a scaffold’s porous structure and its biological properties. To this end we used proprietary 3D Precision Microfabrication Technology to fabricate a biomimetic polycaprolactone scaffold with well controlled porous structures that are optimized for bone and cartilage regeneration and/or repair. To further render desired biological properties to the porous polymer scaffolds, we are further developing a bio-mimetic coating process to coat the porous scaffolds with bone matrix-like extracellular matrix (ECM) by culturing osteogenic cells (including normal and genetically engineered cells). Living cells and residual DNAs are removed after the ECM coating process. These biomimetic scaffolds will have a cell derived ECM coating enriched with proper growth factors, such as BMPs, to recruit stem cells to defect sites for tissue repair. These novel hybrid biomimetic scaffolds are tissue equivalents that can be mass produced without the risk of disease transmission and immune responses that are associated with allograft. These biomimetic scaffolds can be terminally sterilized and ready to use. We believe that this biomimetic coating process can be used to create superior and ready-to-use tissue equivalents for bone regeneration/repair.

Methods

Cells
Human mesenchymal stem cells (hMSCs) and human dermal fibroblast cells (hFB) were purchased from Lonza (Walkersville, MD) and LifeLine Cell Technologies (Walkersville, MD), respectively. According to manufacturer’s instructions, hMSCs and hFB were maintained in growth media. Cells were maintained in a humidified tissue culture incubator at 5% CO2 and 37°C.

Polycaprolactone (PCL) Scaffold Microfabrication (3D Insert™-PCL)
Porous polycaprolactone (PCL) scaffolds were engineered using 3D Biotek’s Proprietary 3D Precision Microfabrication Technology (Figure 1A, B). Uniquely, fiber diameter is controlled by nozzle diameter while spacing between fibers is controlled by a motion control system. The nituses of each layer are oriented 90° relative to the nituses of the layer immediately below (Figure 1B-C). Before use, scaffolds are tissue culture surface treated and x-ray radiation sterilized. This study implemented 30-60-850 compatible 3D Insert™-PCL scaffolds of 5 mm in diameter, 1.5 mm in thickness, and a configuration of 300 µm fiber diameter and 300 µm pore size (PCL3030). The total cell growth area of a 9-well 3D Insert™-PCL3030 is 3.3 cm² compared with 0.25 cm² of total growth area in a traditional 96-well tissue culture plate (TCP).

Biomimetic ECM Coating Process

3D Cell Seeding
hFB & hMSC were statically seeded onto 3D Insert™-PCL scaffolds for 24 h according to 3D Biotek’s 3D cell seeding protocol. A suspension was slowly pipetted onto the top surface of each 3D Insert™-PCL. To ensure high seeding efficiency, the cell suspension droplet was not allowed to contact the sides of the wells. After a 3 h incubation at 5% CO2 at 37°C, 180 µl of media was added to the wells containing 3D Insert™-PCL scaffolds.

Stem Cell Differentiation
Twenty-four hours after seeding, hMSC stem cell growth media was replaced with osteoblastic differentiation media. hMSC osteoblastic differentiation was performed according to manufacturer’s instructions. Every second day, hMSC and hFB cells were replenished with fresh hMSC osteogenic differentiation and hFB growth media, respectively.

Alkaline Phosphatase Activity Assay
hMSC sipes were prepared using M-Per (Pierce) followed by a centrifugation at 14,000 rpm for 5 min. The lysate in supernatant was collected and analyzed using p-Nitrophenyl Phosphate Liquid Substrate Kit (nPP) (SIGMA) and 4-nitrophenol solution. Alkaline phosphatase activity was normalized to DNA concentration.

Scanning Electron Microscopy

Scanning electron micrographs show that week 4 hMSC-osteoc and hFB cells grow along scaffold fibers and extend across pores. SEM analysis demonstrates that only hMSC-osteoblasts form nodules containing calcium and phosphate (Figure SC, D), whereas hFB (Figure 5A, B) form 3D akin-like structures. Further, analysis of week 4 cultures of hMSC-osteoc seeded onto lyophilized week 4 fibrillar- and osteoblastic-derived ECM coated PCL scaffolds (Figure 5E-H) also demonstrated nodule development containing calcium and phosphate on both scaffolds, indicating that both hFB and hMSC-osteoblasts support effective stem cell differentiation into the osteoblastic lineage. Finally, cell growth and morphology of freshly seeded hMSCs into decellularized fibrillar- and osteoblastic-derived ECM coated porous PCL scaffolds was monitored in real-time with a light microscope.

Von Kossa Staining
Scaffolds containing hMSC-osteoc and hFB cells were fixed with 10% formalin for 0.5 h. Cultures were rinsed with ddH2O, incubated with 2% silver nitrate, and covered. Scaffolds were incubated for 10 min and then rinsed again with ddH2O. Exposed to bright light for 15 min, dehydrated in 100% ethanol for 1 min, and dried. Scaffolds with cells were imaged using a digital camera.

Biomimetic Coating Development
Scanning electron micrographs show that week 4 hMSC-osteoc and hFB cells grow along scaffold fibers and extend across pores. SEM analysis demonstrates that only hMSC-osteoblasts form nodules containing calcium and phosphate (Figure SC, D), whereas hFB (Figure 5A, B) form 3D akin-like structures. Further, analysis of week 4 cultures of hMSC-osteoc seeded onto lyophilized week 4 fibrillar- and osteoblastic-derived ECM coated PCL scaffolds (Figure 5E-H) also demonstrated nodule development containing calcium and phosphate on both scaffolds, indicating that both hFB and hMSC-osteoblasts support effective stem cell differentiation into the osteoblastic lineage. However, hMSC-osteoc ECM promoted faster mineral node formation as compared to hFB ECM coating.

Discussion
This study demonstrates that it is possible to create an ideal scaffold with desired porous structure and bioactivity through a combination of 3D Precision Microfabrication and a cell culture biomimetic coating processes. Specifically, a bone matrix-like ECM can be applied to a scaffold to create a tissue equivalent for bone repair/regeneration. hMSCs that proliferate and differentiate into the osteoblastic lineage on these PCL scaffolds generated a uniform, cell-derived bone matrix-like ECM coating that is richly incorporated with osteoinductive and osteoconductive substances.

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