

## NIST Reference Material Scaffolds Characterized for Cell Response

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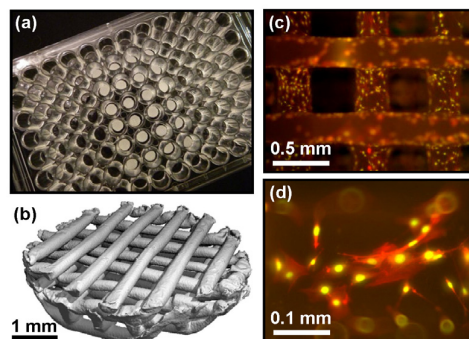
**Statement of Purpose:** Reference scaffolds characterized for cell response are being developed for use as a standard for biological characterization of new scaffolds. The need for reference scaffolds to serve as a calibration standard between labs has been identified as critical to advancing tissue engineering science [1,2]. The “Regenerative Medicine Promotion Act” was recently introduced into the U.S. House of Representatives which specifically calls for NIST to develop standards for regenerative medicine products [3]. Previously, NIST deployed reference scaffolds with well-characterized structure and porosity (Fig. 1) [4]. The current effort expands the scope to include cell culture data for morphology, attachment and proliferation. Freeform fabrication (FFF) was chosen to make the reference scaffolds since this technique affords precise control of scaffold structure. Poly( $\epsilon$ -caprolactone) (PCL) was chosen as the polymer since it is used in biomedical implants and is being investigated for tissue scaffold applications. MC3T3-E1 osteoblasts were used for cell response since scaffolds are frequently used for bone tissue engineering. 96-well plate scaffolds were selected for efficiency (fewer reagents, lower cost).



Fig. 1. First generation NIST reference scaffolds currently available and characterized for structure (strut diameter, strut spacing, porosity).

**Methods:** Freeform fabricated scaffolds were from 3D Biotek, LLC. They were made by precision extrusion deposition in a 0°/90° lay-down pattern (“log cabin”). Each scaffold fits in a 96-well and a “unit” is 24 scaffolds in a 96-well plate. Scaffolds were cylindrical with the following properties (determined by microscopy, gravimetry and X-ray tomography): 5.3 mm diameter, 1.6 mm thickness, 0.3 mm strut diameter, 0.5 mm “x-y direction” strut spacing, 0.2 mm “z-direction” strut spacing and 60% porosity. Scaffolds were made from PCL (relative molecular mass 53,000 g/mole). MC3T3-E1 murine osteoblasts (Riken cell bank) were cultured in  $\alpha$ -MEM with 10% fetal bovine serum and 0.06 mg/mL kanamycin sulphate. Passage 3 cultures at 80% confluency were used for experiments (20,000 cells seeded per well). Each experiment used one “unit” where 3 scaffolds were used for fluorescence microscopy and 6 were used for a DNA assay at each of two time points (1 d and 7 d) using a total of 18 scaffolds (leaving 6 extra scaffolds per “unit”). The same experiment was performed three times over 3 successive weeks (1 per week) using separate “units” of scaffolds to test

repeatability. For fluorescence microscopy, cells were fixed (3.7 % formaldehyde in PBS, 1 h), permeabilized (0.2 % Triton X-100 in PBS, 5 min) and stained 1 h in PBS with Alexa Fluor 546 phalloidin (33 nM, red actin) and Sytox green (1  $\mu$ M, green nuclei). Stains were from Invitrogen. The Picogreen DNA assay (Invitrogen) was used to assess DNA content. Cells on scaffolds were incubated in lysis buffer overnight (0.2 mg/mL Proteinase K and 0.02 % by mass sodium dodecyl sulphate in Tris-EDTA buffer). DNA assay was performed according to manufacturer’s instructions using a DNA standard curve.



(e) Picogreen DNA Assay

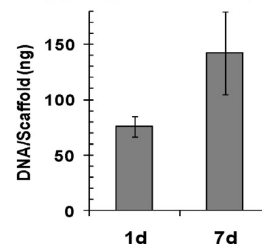


Fig. 2. (a) Photograph of 24 scaffolds in a 96-well plate. (b) X-ray tomography of a single scaffold. (c-d) Fluorescence micrographs of osteoblasts cultured 1 d on scaffolds (red = actin, green = nuclei). (e) DNA assay for cell number on scaffolds after 1 d or 7 d culture (n = 3, error bars are S.D.). T-test indicates 1 d is different from 7 d (P < 0.05).

**Results & Conclusions:** Osteoblasts adhered, spread and proliferated on reference scaffolds in a repeatable manner (Fig. 1) indicating that they can be used as reference materials for in vitro cell culture testing.

**Notes:** The “standard deviation” (S.D.) is the same as the “combined standard uncertainty of the mean” for the purposes of this work. This article, a contribution of NIST, is not subject to US copyright. Certain equipment and instruments or materials are identified in the paper to adequately specify the experimental details. Such identification does not imply recommendation by NIST, nor does it imply the materials are necessarily the best available for the purpose.

### References:

1. Multi-Agency Tissue Engineering Science (MATES), Report: Advancing Tissue Science and Engineering, 2007.
2. FP7 Work Programme, 2.3-1 Development of standard scaffolds for the rational design of bioactive materials for tissue regeneration, European Commission, 2009.
3. H.R. 6173 Regenerative Medicine Promotion Act, introduced into U.S. House of Representatives, September 22, 2010.
4. NIST Reference Materials 8395, 8396, 8397, Report of Investigation for Tissue Engineering Reference Scaffolds, 2009.