Supporting Information

Development of Injectable Citrate-Based Bioadhesive Bone Implants

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MC3T3 cells proliferation and differentiation on iCMBA/HA composites

To evaluate the cytocompatibility of iCMBA/HA composites, the proliferation and differentiation of MC3T3 (MC3T3-E1 SUBCLONE 4 (ATCC CRL®-2593™)) on iCMBA/HA composites were studied. The viability and adhesion of MC3T3 cells to the surface of iCMBA/HA composites were studied using fluorescent microscopy. Briefly, about 20 μL iCMBA/HA mixture (iCMBA-$P_{200}D_{0.3}$ PI:8%-HA70% (iCH70)) (prior to completion of cross-linking) was uniformly spread on the surface of a glass slip cover to form a thin layer of the crosslinked composite. The samples were then sterilized by incubation in 70% ethanol for 24 hours followed by exposure to UV light for 3 hours. The samples were then placed in 24-well plate and seeded with MC3T3 cells with a density of 50,000 cells/cm^2 followed by adding complete MEM media (Minimum Essential Medium (MEM) Alpha cell culture medium (Invitrogen Corp, Eugene, OR), containing 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) streptomycin). At each time point (day 1 and day 3 post cell seeding) the constructs were removed from the well plate, rinsed by PBS and stained with CFDA-SE (Carboxyfluorescein diacetate, succinimidyl ester) dye (Vybrant® CFDA SE Cell Tracer Kit, Invitrogen Corp, Eugene, OR) as per manufacturer's procedure. Next, the

fluorescent images of stained samples were taken using a Nikon Eclipse Ti-U microscope equipped with Andor DR-328G camera (Nikon Instruments Inc, Melville NY).

The proliferation of MC3T3 cells on the surface of iCH70 composites were determined by DNA assay. The composite samples were cut in disk shape to fit into the 24-well plate, and sterilized by incubation in 70% ethanol for 24 hours followed by exposure to UV light for 3 hours. MC3T3 cells were then seeded on the composites at a density of 50,000 cells/cm^2 followed by adding 1 ml of complete MEM media. At each time point, the samples were rinsed with TBS 1X (Tris-buffered saline), underwent 3 cycles of freeze-thaw-sonication in order to break cells and expose their DNAs. Quant-iT™ PicoGreen ® dsDNA reagent (Invitrogen Corp, Eugene, OR) was used to quantify the double-stranded DNAs. Using a known quantity of cells, a standard curve was also prepared as a reference to correlate between fluorescence intensity and number of cells.

The differentiation of MC3T3 osteoblast precursor cells to osteoblasts was tracked by measuring the activity of alkaline phosphatase (ALP) produced by osteobalsts. Cell-composite constructs were then prepared as previously described. Briefly, MC3T3 cells were cultured on sterile composite samples and control cell culture plate at a seeding density of 50,000 cells/ cm^2. Twenty-four hours after seeding, the cell culture media was replaced by a differentiation media containing 50 µg ascorbic acid and 3.06 mg of beta-glycerol phosphate (BGP) in 1 mL of complete MEM media. The differentiation media was replaced every other day. At each time point the constructs were washed with PBS and went through 3 freeze-thaw-sonication cycles. ALP activity was then measured through incubation of 50 µL aliquots of homogenates with 4-nitrophenyl phosphate solution at 37^oC for 30 minutes. The amount of 4-nitrophenyl generated due to the presence of ALP was then measured through absorption at 405nm using spectrophotometer and referenced to a standard curve of 4-nitophenyl. ALP activity was normalized to the number of cells at each time point, which was separately measured by DNA assay.

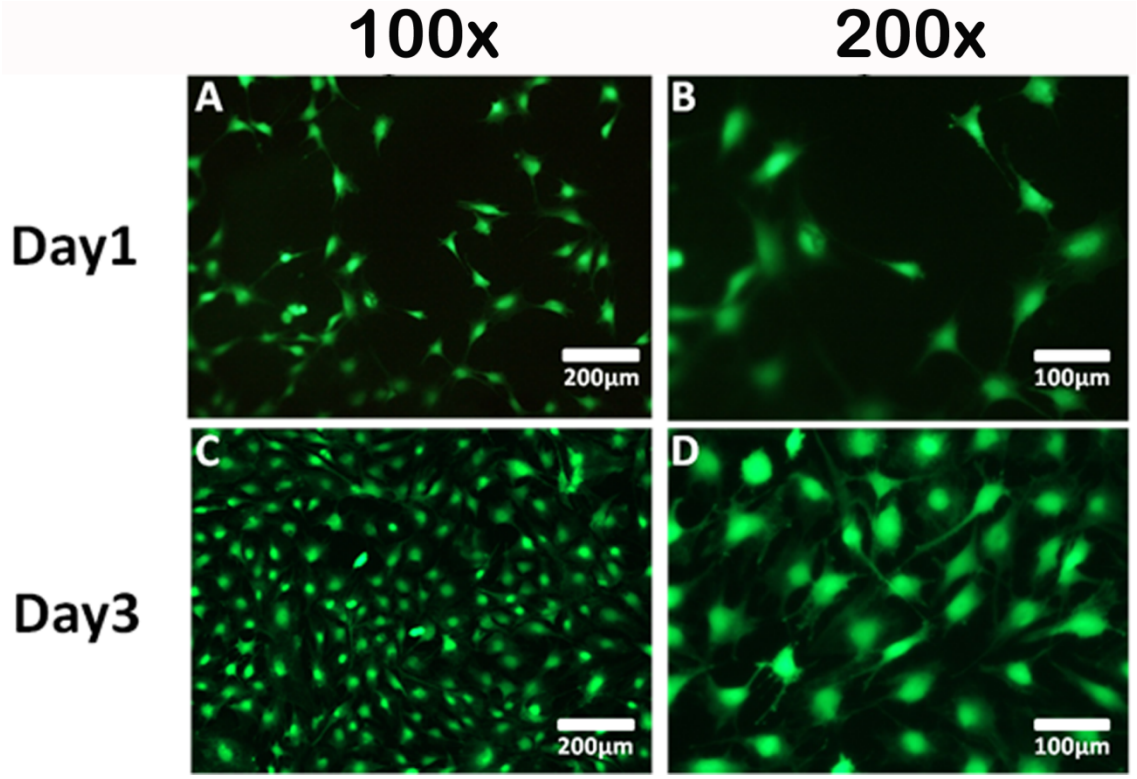

Fig. S1. Fluorescent images of CFDA-SE stained MC3T3 pre-osteoblast cells seeded on the iCMBA-$P_{200}D_{0.3}$ PI:8%-HA70% (iCH70) composite films at day 1 (A, B) and day 3 (C, D) post-seeding.

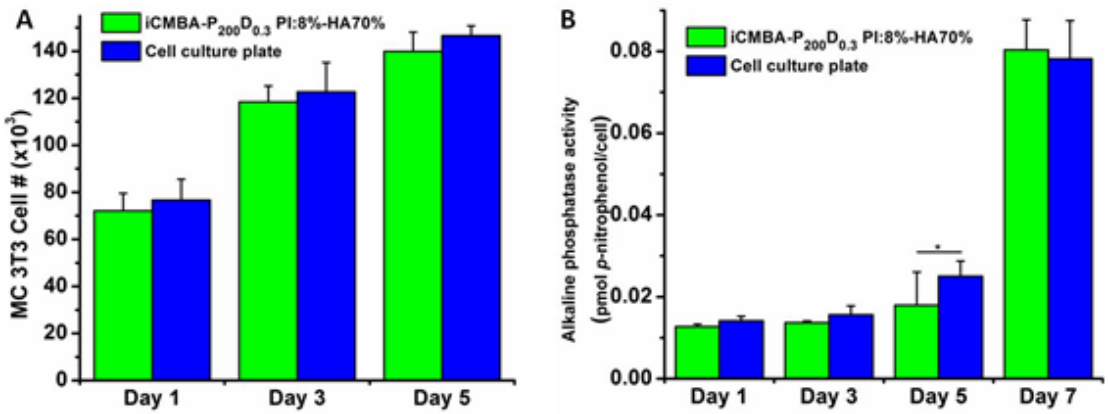

Fig. S2. MC3T3 pre-osteoblast cells proliferation and differentiation on the iCMBA-$P_{200}D_{0.3}$ PI:8%-HA70% (iCH70) composite and cell culture plate (control). A) Proliferation of the cells in growth media measured through PicoGreen DNA assay at 1st, 3rd, and 5th day post seeding. B) Differentiation of pre-osteobalst cells to osteoblasts measured by ALP expression at 1st, 3rd, 5th, and 7th day post adding the differentiation medium.