Biological Characterization of TAMP Scaffolds for Hard Tissue Regeneration

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Abstract

Tissue engineering provides a better alternative to implant materials on the market today, which do not promote cell-material interaction and frequently result in scar formation around the implant causing a limited life span of the material. An ideal bioengineering material promotes cell-material interaction and mimics the structural features of the tissue to be regenerated. Recently, we have developed technology for fabricating novel, nano-macroporous bioactive 'tailored amorphous multi-porous' (TAMP) scaffolds of composition 30 mol%CaO - 70 mol%SiO through a novel sol-gel process (Marques et al. 2009). It has shown excellent bio-compatibility via the rapid formation of hydroxyapatite in simulated body fluid (Vueva et al. 2010), as well as in early tests with bone forming cells (Wang et al. 2011). Here we report a detailed investigation of the response of MC3T3-E1 pre-osteoblasts to these TAMP scaffolds. Our results show that MC3T3-E1 pre-osteoblast cells adhere, proliferate, colonize, and differentiate on and inside bioactive TAMP scaffold supporting their usefulness as a material for tissue engineering.

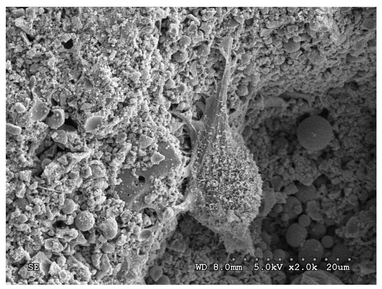

Figure 1: SEM image of a cell adhering & spreading on a TAMP scaffold 1hr after seeding.

Methods

TAMP scaffolds were prepared as indicated in Figure 2. They were then polished, washed, autoclaved, and pre-incubated in PBS for 2-3 days before cells were seeded on them. At each appropriate time point, scaffolds with cells were fixed and processed for immunofluorescence or Scanning Electron Microscopy (SEM), mRNA was collected to make cDNA for qRT-PCR analyses, or a cell lysate was prepared for use in the Alkaline Phosphatase assay (ALP) which was normalized to total protein determined by a Bradford Colorimetric Assay (BCA).

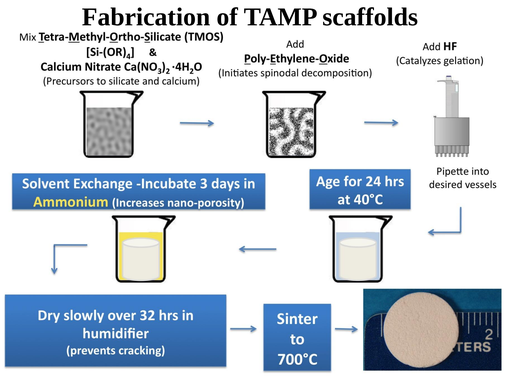

Figure 2: Our novel Sol-gel process allows us to produce 30 mol%CaO – 70 mol%SiO scaffolds with nanopores, important for fluid exchange and surface area, and interconnected macropores which are large enough to allow cells to enter and grow inside the TAMP scaffolds.

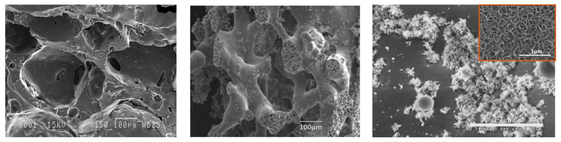

Figure 3: SEM comparison of trabecular bone (organic compounds removed) (left) to TAMP scaffold (middle) shows similar morphologies. After 3 to 10 days in medium or simulated body fluid, scaffolds form hydroxyapetite indicating that it is bioactive.

Results & Discussion

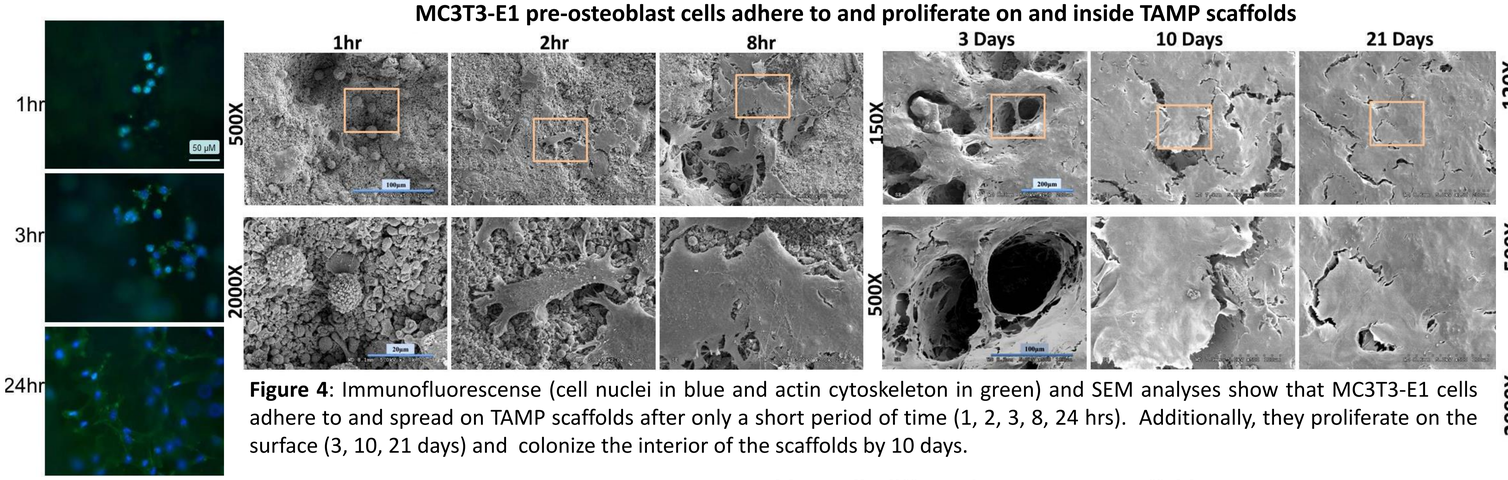

Figure 4: Immunofluorescense (cell nuclei in blue and actin cytoskeleton in green) and SEM analyses show that MC3T3-E1 cells adhere to and spread on TAMP scaffolds after only a short period of time (1, 2, 3, 8, 24 hrs). Additionally, they proliferate on the surface (3, 10, 21 days) and colonize the interior of the scaffolds by 10 days.

MC3T3-E1 pre-osteoblast cells differentiate on TAMP scaffolds

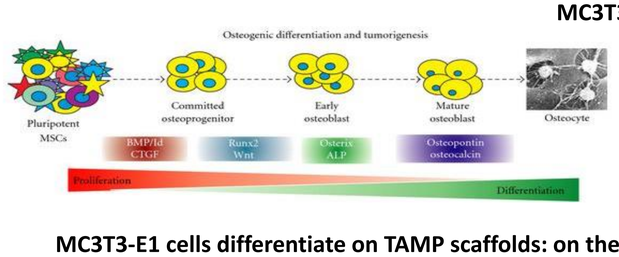

Figure 5: Schematic representation of temporal gene expression in bone cell differentiation (Wagner et al. 2011). MC3T3-E1 pre-osteoblasts are lineage committed precursors to bone cells. These cells have been well characterized, and it is known that as they differentiate into osteoblasts a specific temporal trend in gene expression occurs. We are investigating these changes in gene expression to determine if the TAMP scaffolds are inducing differentiation.

MC3T3-E1 cells differentiate on TAMP scaffolds: on the mRNA level

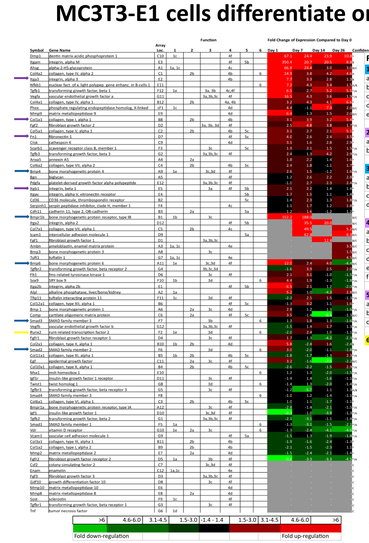

Figure 6: 96-well bone cell differentiation array (SA-Biosceinces/Qiagen) shows the early up-regulation of BMPs, their receptors, and SMADs, which supports the activation of a BMP-mediated differentiation pathway in MC3T3-E1 osteoblast precursor cells grown on sol-gel derived scaffolds. Importantly, RunX2, the 'master regulator' of bone cell differentiation, is up-regulated on day 7 in a timely manner. Extracellular matrix proteins (collagen, fibronectin) and their receptors (integrins) are constitutively expressed. Collectively, these findings support a TAMP scaffold-mediated differentiation of pre-osteoblasts into mature osteoblasts.

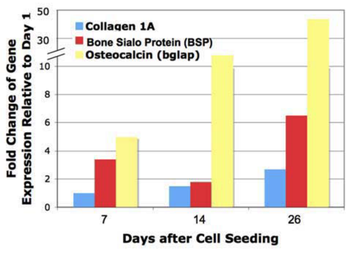

Figure 7: qRT-PCR analyses of bone specific proteins not included on the 96-well array indicate that on the mRNA level MC3T3-E1 cells growing on TAMP-scaffolds differentiate into mature bone secreting osteoblasts. Replicates of six samples were analyzed using sets of custom designed oligonucleotides.

MC3T3-E1 cells differentiate on TAMP scaffolds: on the protein level

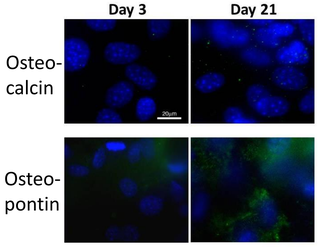

Figure 8: MC3T3-E1 cells grown on TAMP scaffolds for 21 days secrete bone specific extracellular matrix proteins indicating differentiation. (Cell nuclei stained blue and bone specific proteins (osteopontin and osteocalcin) stained green).

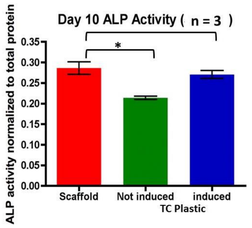

Figure 9: MC3T3-E1 cells growing on scaffolds have significantly (as indicated by the *) higher Alkaline Phosphatase activity on day 10 than cells growing on tissue culture plastic. Yet comparable enzyme activity was measured for cells growing on scaffolds and positive control cells that were induced to differentiate, indicating cell differentiation on the scaffolds. (These data have not been corrected for BSA absorbed to TAMP scaffolds.)

Conclusions

Our results show that MC3T3-E1 pre-osteoblast cells adhere, proliferate, colonize, and differentiate on and inside bioactive TAMP scaffolds. Concomitant experiments investigate how to tailor the scaffolds to the specific needs of the regenerating tissue, the role of nanopore size in cell attachment, and how cells are sensing the scaffold.

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References

Marques, A.C. et al., J. Mater. Res., 2009, Vol. 24, No. 12,. 3495-3502.
Vueva, Y. et al., J. Am. Ceram. Soc. 2010. Vol. 93(7).
Wagner et al., Sarcoma, 2011, Vol 2011, 1-12.
Wang, S. et al. J Mater Sci: Mater Med. 2011. Vol. 22:1195–1203.