

Scaffolds and Cells for Bone Engineering

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INTRODUCTION: Functional scaffolds are essential for some bone tissue engineering approaches. In many cases the ideal scaffold should be biodegradable at a rate which compliments the ingrowth of host tissue. Specifically, bone engineering scaffolds should also be osteoconductive so that, as the scaffold degrades, the structural integrity of the composite will be maintained. Our approach has been to develop a polymeric based biodegradable material with a macroporous, interconnecting, structure which mimics the dimensional scale ranges found in human trabecular bone. This scaffold has evolved in an iterative manner to respond to the biological reactions to the scaffold. These biologically-driven design criteria have resulted in a novel 3-phase biodegradable, anti-inflammatory, and osteoconductive scaffold material, on which we can seed human osteoprogenitors. We source the latter from either adult marrow – mesenchymal progenitor cells (MPC) or by extraction of perivascular cells from human umbilical cord (HUCPV), to provide both autogeneous and potentially allogenic tissue engineering strategies.

METHODS: Macroporous, biodegradable poly(lactide-co-glycolide) (PLGA) scaffolds containing an osteoclast-resorbable calcium phosphate particulate phase were fabricated by a salt-leaching phase inversion technique which we have described in detail elsewhere (1). In some cases these scaffolds were coated with a thin film of CaP by immersion in 5 x simulated body fluid (SBF) for 5days.

Adult human marrow cells were obtained from marrow discarded from marrow transplant donors. The harvest was elutriated using a Ficoll-Paque™ (Sigma) density gradient to recover the mononuclear cell fraction. The cells were inoculated (~1 x 10⁶ cells/ml) into siliconized stirred suspension spinner flasks with medium containing 2 ng/ml and 10 ng/ml of highly purified recombinant human IL-3 and SCF respectively (2). The cell population expanded in these non-contact conditions was then plated on culture substrates to conduct bone nodule (CFU-O) assays. Some cells were seeded directly, from suspension, into scaffolds.

HUCPV were obtained from consenting full-term caesarian section patients. The umbilical cord was sectioned and the amniotic epithelium opened to reveal the vessels which were removed together with their peri-vascular tissue. These vessel fragments were then looped and the perivascular tissue enzymatically digested in 0.5mg/ml collagenase in PBS. The resulting cell harvest was cultured in both non-osteogenic and osteogenic (dexamethasone-containing) culture conditions.

RESULTS & DISCUSSION: The 3-phase biodegradable scaffold has evolved, in an iterative manner, to address several problems associated with the biological response to such compliant materials. The mechanical properties of the native PLGA geometry are insufficient to withstand the contractile forces of high densities of seeded cells. This is overcome by including a calcium phosphate particulate phase. The resulting scaffold has sufficient mechanical strength but is still not osteoconductive. The latter is achieved by the second calcium phosphate coating phase.

The cell sources provide, in the case of MPC, a rapidly expanding mesenchymal progenitor population which maintains a functional phenotype when finally seeded on a solid substrate and produces morphologically identifiable bone matrix. The HUCPV cells represent a rapidly expanding population of adherent cells which contain a subpopulation that exhibits osteogenic potential even in non-osteogenic culture conditions. Furthermore these cells, upon storage and passage, show an increasing population of MHC-/- cells, as determined by flow cytometry, and thus represent a potential allogeneic source of human cells for tissue engineering and other cell-based therapeutic strategies.

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References: (1) Holy CE et al. (2003) J Biomed Mater Res. 65A(4): 447-53. and Guan L and Davies JE (2004) Preparation and characterization of a highly macroporous biodegradable composite tissue engineering scaffold. *Submitted* (2) Baksh D, Davies JE, Zandstra PW 2003 Exp. Hematol. 31:723-732. and Baksh D, Zandstra PW, Davies JE, (2004) Osteogenic development from long-term, non-adherent, suspension cultures of adult human bone marrow-derived cells *Submitted*