Three-Dimensional Culture and Differentiation of Stem Cells for Regenerative Medicine

Expansion of stem cells (SCs) including pluripotent embryonic stem cells (ESCs) and multipotent mesenchymal stem cells (MSCs) using two-dimensional (2-D) culture conditions is laborious and technically challenging. We hypothesized that culturing SCs in three-dimensional (3-D) scaffolds could maintain long-term self-renewal and differentiation potential. The goal of our research was to develop self-assembling scaffolds that support self-renewal or differentiation of SCs in 3-D culture. The specific aims were to: 1. investigate self-assembling and prefabricated scaffolds for growth and differentiation of mouse ESCs; 2. optimize components and assembly of scaffolds that support expansion of human ESCs; and 3. determine feasibility of *ex vivo* culturing and differentiation of MSCs to treat degenerative disc disease (DDD).

Polyethylene glycol and dextran were functionalized with acrylate and/or thiol reactive end groups to design self-assembling scaffolds by a thiol-Michael addition reaction. The functionalized polymers mixed with cells self-assembled while encapsulating cells in the presence of oxygen. These scaffolds were incubated in growth medium for cell proliferation and monitored using light microscopy and MTT analysis. Comparative transcriptional and translational analyses of SCs grown in 3-D and traditional 2-D culture conditions were performed using qRT-PCR and immunocytochemistry. Pluripotency of ESCs was determined by inducing germ layer differentiation *in vitro* and teratoma formation *in vivo*.

3-D scaffolds supported long-term pluripotent growth of ESCs, which exhibited morphology and differentiation potential comparable or better than 2-D culture. Interestingly, ESCs expressed core (OCT4, NANOG, and SOX2) and naïve (KLF4, KLF17, TFCP2L1, DPPA3, and DNMT13L) pluripotency markers at significantly higher levels in 3-D than 2-D cultured ESCs suggesting that scaffolds were a more conducive pluripotent environment. In MSCs, self-assembling scaffolds induced differentiation into chondrogenic cells, which expressed nucleus pulposus (NP) markers, FOXF1, K19, and OVOS2. MSCs encapsulated in self-assembling scaffolds produced NP components *ex vivo* and could be used for treating DDD.

In conclusion, we have developed novel self-assembling scaffolds for maintenance and expansion of ESCs and differentiation of MSCs into large numbers of uniform cell populations. The robust and reproducible 3-D culture methods developed in our studies will not only advance the field of stem cell research but also regenerative medicine applications.