Quiz #1 Answers

1. (30%) In a certain disease condition it is necessary to remove a portion of degenerated annulus fibrosus and nucleus pulposus (procedure called discectomy; Fig. 1c).
   a. What fabrication method would you use to produce a 1-piece scaffold if it were to induce the architecture of the tissues in the defect in Fig. 1c?
      The pore structure (e.g., pore orientation) for the portions of the implant in the annulus fibrosus (AF) and nucleus pulposus (NP) would have to be different in order to induce the native architecture. It might be useful to fabricate the scaffold with a lamellar architecture for the portion of the implant to be in the AF region. The best fabrication method would likely be a free-form fabrication method, such as 3-D printing, in order to obtain precision control over the structure/porosity of the scaffold.
   b. Note the principal limitation of this fabrication method?
      A principal limitation of free-form fabrication methods is only certain types of materials can be utilized.
   c. What would you expect as the outcome of implanting a sponge-like scaffold (alone) into the defect, regardless of how it is fabricated?
      The portion of the implant in the NP would not become infiltrated with cells because the chondrocytes of the NP are surrounded by their extracellular matrix, and thus not free to migrate. Moreover, there is a low cell number density in the NP. In addition, because the NP is non-vascular, there would be no blood-borne cells in the scaffold in the NP region.
      AF cells would likely migrate into the scaffold in that region of the implant and the bleeding from the vascular tissue may bring blood-borne cells into the scaffold.
   d. What benefits might there be in drilling small holes into the bone above and below the defect, prior to implanting the scaffold alone?
      Performing “microfracture” though the endplates of the adjacent vertebrae would introduce blood and marrow into the scaffold. Cells from the marrow (including mesenchymal stem cells) might contribute to a reparative response in the defect.

2. (30%) One of the main projects in your company is the production of tissue-engineered nucleus pulposus in vitro. There are questions regarding the most favorable cells and culture conditions to use for the formation of the nucleus pulposus construct in culture.
   One proposition is that chondrocytes be isolated from the patient’s degenerative nucleus pulposus removed during discectomy. Another approach is that marrow be aspirated from the patient for the isolation of mesenchymal stem cells, and that these stem cells be differentiated to chondrocytes in vitro.
   a. Which of the 2 cell types would you select for the tissue-engineered cartilage?
      The benefit of the MSCs is that when they differentiate to a specialized cell type like a chondrocyte, the cell has the characteristics of a young cell, even though the MSC might be
derived from the marrow of an older individual. Cells from the degenerative nucleus pulposus tissue may be compromised.

b. It has been suggested by the CEO that the application of any type of mechanical loading to the cartilaginous construct as it forms in vitro will be of some benefit, and she has proposed the cheapest approach of applying a constant load to the cartilage as it forms. Do you agree?
   Do not agree. A constant load can stimulate the production of proteolytic enzymes, i.e., an adverse effect. Alternatively, a cyclic (dynamic) compressive or shear load will stimulate the cells to produce matrix molecules (a beneficial effect).

c. At what stage of maturation would you propose that the tissue-engineered nucleus pulposus construct be implanted?
   An immature construct may be better incorporated into the host tissue, but be susceptible to breakdown under the mechanical loading. A mature scaffold may be able to sustain the mechanical loading but may not undergo remodeling necessary for integration into the host tissue and to impart the stress-induced architecture necessary for long term performance.

3. (30%) Due to trauma, fissures/defects can form in the nucleus pulposus, annulus fibrosus, and/or in the bone (Fig. 1d). The company is considering the development of an injectable treatment for each of these 3 defects.
   a. For each of the 3 defects select one of the following, and explain the basis of your choice: 1) no treatment required; 2) an injectable self-assemblying peptide; 3) cells of the same type as the tissue; 4) cells of the same type as the tissue incorporated in the self-assemblying peptide. Choose the simplest solution.
      Bone: No treatment required; the bone will likely spontaneously regenerate in the defect.
      Annulus Fibrosus: An injectable self-assemblying peptide; cells from the AF may migrate into the scaffold.
      Nucleus Pulposus: Cells of the NP incorporated into the self-assemblying peptide.

   b. One of the consultants to your company has proposed that the three defects can simply be treated by giving the patient a systemic injection of drug known to stimulate the proliferation of mesenchymal stem cells in marrow and their release to the general circulation. Do you agree for each of the defects?
      The MSCs in the circulation will migrate to the bone and AF defects and become engrafted to the lesions. However, because it is non-vascular, the MSCs from the circulation would not reach the defect in the NP.

   c. For the treatment of the defect in the nucleus pulposus, another consultant has suggested the use of embryonic stem cells derived from somatic cell nuclear transfer which have been differentiated to chondrocytes in vitro. Ethical issues aside, do you agree?
      The potential problem is that some undifferentiated embryonic stem cells may be in the cell suspension that is injected into the patient, and these undifferentiated cells could form a teratoma.

4. (10%) Your company has technology to produce sponge-like scaffolds of the same pore structure, but with different mechanical rigidity (hardness). If you were asked to use
mesenchymal stem cell-seeded scaffolds for implantation into defects in the nucleus pulposus, annulus fibrosus, and bone, would this technology be of any value? [These would be non-injectable applications for larger defects than that shown in Fig. 1d.]

The mechanical stiffness of a substrate can affect the differentiation of MSCs. Stiff substrates result in the differentiation of MSCs to osteoblast-like cells. While it has not yet been proven, cells of a more compliant/softer scaffold may be found to favor the differentiation of the MSCs to chondrocytes. Scaffolds of still other mechanical properties may be favorable for differentiation of MSCs to AF cells.