

Biomaterials - Tissue Interactions

Quiz #1

1 (80%). Implants to Treat Defects in the Intervertebral Disc.

The intervertebral discs (IVD) of the spine are sandwiched between the bone vertebrae (Fig. 1a). The IVD is made up of an outer “annulus fibrosus” comprised of fibrous tissue with a lamellar structure in which the collagen fibers in each lamella are oriented in a particular angle (Fig. 1b). Contained within the annulus fibrosus, making up the core of the disc, is the “nucleus pulposus,” which is cartilage (Fig. 1b). The bone and annulus fibrosus are vascularized but the nucleus pulposus has no blood vessels. You have been hired by an orthopaedic company interested in developing novel treatments for spine problems.

A. (10%) Due to trauma, small fissures/fractures/defects can form in the nucleus pulposus, annulus fibrosus, and/or in the bone (Fig. 1c). Write 3 interconnected unit cell processes, involving 3 different cell types, for the acute phase of healing for each of the 3 defects. Name a regulator for each process if one is known.

B. (15%) The company is considering the development of treatments for each of the 3 small defects in Fig. 1c. Which of the elements of tissue engineering/regenerative medicine (scaffolds, cells, regulators), if any, would you suggest are the most essential for implantation or injection into each of the 3 defects to facilitate the most favorable reparative response. You can name more than one element, but there must be a compelling rationale. Explain your answers.

C. (15%) In certain disease conditions it is necessary to remove a portion of degenerated annulus fibrosus and nucleus pulposus, in a procedure referred to as discectomy (Fig. 1b). One proposed method of treatment is to implant a permanent, nonporous silicone rubber device which has the same shape as the defect and compressive properties that are similar to the resected tissue.

(1) What are two principal issues which would have to be addressed relative to this approach?

(2) When this procedure was performed in an animal model it was found that a layer of macrophages was on the silicone samples when they were retrieved after 6 months. One of your colleagues has notified the CEO that this is cause to search for another elastomeric polymer for this application, because the presence of the macrophages indicates that the silicone rubber being used is not biocompatible. Do you agree? Explain.

(3) When the silicone sample (above) was removed from the animal it was found to be encapsulated in a layer of tissue a few millimeters thick. Write the unit cell process(es) related to the formation of this tissue capsule.

D. (20%) As an alternative approach to the treatment of the defect in Fig. 1b, your company has developed a sponge-like absorbable scaffold to fill the defect to facilitate regeneration. The least expensive approach would be to have a one-piece sponge-like scaffold fill the defect.

(1) What would you expect the outcome of this procedure to be relative to the growth of nucleus pulposus and annulus fibrosus tissue into the scaffold?

(2) Would there be any benefit to applying laminin on the walls of the scaffold prior to its implantation?

(3) Would there be any benefit to poking holes through the ends of the bone above and below the defect prior to implanting the scaffold?

E. (10%) A prosthesis is being investigated to replace the entire degenerated intervertebral disc, after it is removed (Fig. 1d). The metal endplates of the metal-plastic (polyethylene)-metal device have porous metal coatings to accommodate bone ingrowth for fixation (Fig. 1d).

(1) Write 3 unit cell processes involved in this bone ingrowth into the coating. Name a regulator for each process if one is known.

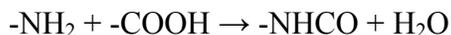
(2) In an animal model, a prosthesis recovered after 6 months of implantation had fibrous tissue, not bone, in the pores of the coating. What is the likely explanation for this finding.

F. (10%) During laboratory mechanical testing of the prosthesis in 1.E. (Fig. 1d), it was found that wear particles were released by the polyethylene. One of your colleagues at the firm has the opinion that these will not prove to be a problem if generated *in vivo* because they are so small (less than 10 μm in diameter) they will not interfere with function of the spine. Do you agree? Explain your answer using unit cell processes.

2 (20%) Loss of Cell-Biomaterial Binding Due to Manufacturing Error during Preparation of Biomaterial.

One of the intermediate steps of the process for manufacturing a collagen-based scaffold subjects type I collagen (extracted from cow tendon) to a freeze-drying step. During this step the aqueous suspension of collagen is first frozen and then dried in the frozen state by sublimation of the ice into the vapor state (following application of vacuum). At the end of the freeze drying step the temperature is 25°C and the water content of collagen has dropped down drastically from about 99% to 5-10% by weight.

Residual moisture is further reduced in the next step, high-temperature crosslinking (the last in the manufacturing process). Here, the combination of high temperature, 104°C, and a vacuum of 50 torr (1 torr = 133Pa) removes moisture from the material from about 5-10% down to less than 1% over a period of several hours. Removal of the residual moisture below about 2% is known to induce covalent crosslinking between chains of collagen. Crosslinking takes place primarily by an amide condensation process involving amino groups and carboxylic groups from adjacent chains, as shown below:



On a particular day the vacuum pump during the freeze drying step fails and the pressure increases to atmospheric. As a result the sublimation process fails and the material does not become dehydrated down to the desired level. The moisture content at the end of this failed freeze drying step is now 30%. The ensuing crosslinking step is conducted under normal conditions. After the crosslinking step it is observed that the product fails the basic quality control test: The scaffold product fails to bind large numbers of fibroblasts on its surface when a small volume of cells in culture is placed on the surface of a sample of the product. In a normal quality control test the cells bind on the surface in very large numbers.

Explain the failure of the quality control test. To support your answer cite (in very brief, summary form) data from one or more of the articles in the reading assignment. When citing data that support your interpretation mention the complete reference, including author, journal, date, as well as the page number where the cited data is located.

NOTE 1: Some data on the thermal transitions (melting to gelatin) of type I collagen.

At a sufficiently high temperature, collagen melts into gelatin. Gelatin molecules have the same secondary structure as collagen does but they lack the tertiary structure of collagen. The temperature of the transition from collagen to gelatin decreases as the moisture content increases. Positive pressure or vacuum at levels near atmospheric pressure do not affect the level of the melting point. Examples of such melting points (T_m , °C) are shown in the Table below:

% moisture	T_m , °C
1	200
10	120
30	90
50	70

NOTE 2: Single-letter amino acid code

1. **G** - Glycine (Gly)
2. **P** - Proline (Pro)
3. **A** - Alanine (Ala)
4. **V** - Valine (Val)
5. **L** - Leucine (Leu)
6. **I** - Isoleucine (Ile)
7. **M** - Methionine (Met)
8. **C** - Cysteine (Cys)
9. **F** - Phenylalanine (Phe)
10. **Y** - Tyrosine (Tyr)
11. **W** - Tryptophan (Trp)
12. **H** - Histidine (His)
13. **K** - Lysine (Lys)
14. **R** - Arginine (Arg)
15. **Q** - Glutamine (Gln)
16. **N** - Asparagine (Asn)
17. **E** - Glutamic Acid (Glu)
18. **D** - Aspartic Acid (Asp)
19. **S** - Serine (Ser)
20. **T** - Threonine (Thr)
21. **O** – Hydroxyproline (Hypro)

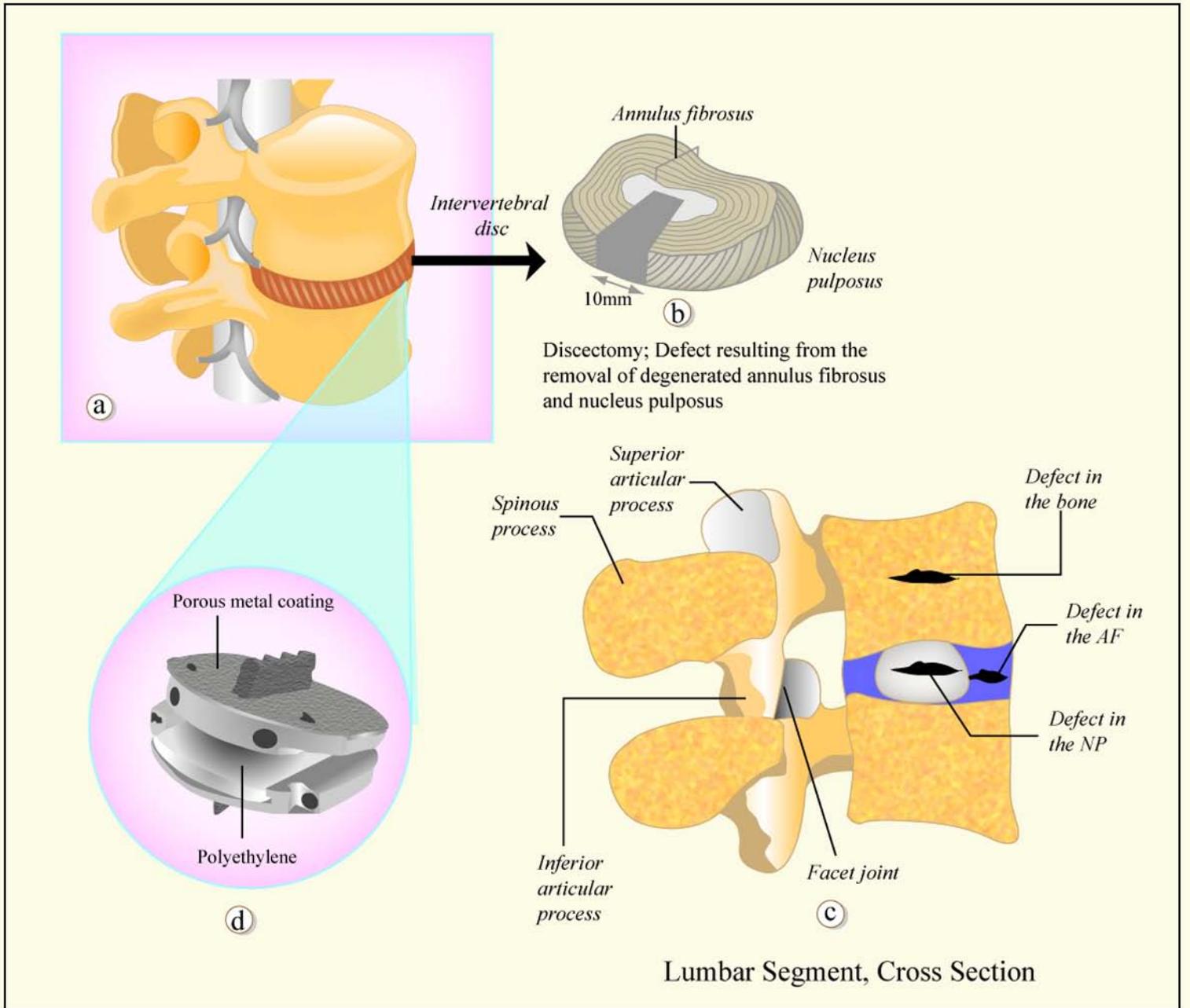


Figure by MIT OpenCourseWare.

Fig. 1

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