1. What kind of cells are used in the Novocart3D treatment for cartilage lesions ?

    a. Autologous mesenchymal stem cells
    b. Autologous induced pluripotent stem cells
    c. Autologous undifferentiated cells
    d. Autologous differentiated cells
    e. Allogenic cells to reduce the risk of disease transfer

In the Novocart 3D approach, cells were isolated from cartilage tissue meaning these were already differentiated into the cell of cartilage (chondrocytes). The biopsy is taken from the patient meaning the cells are autologous.

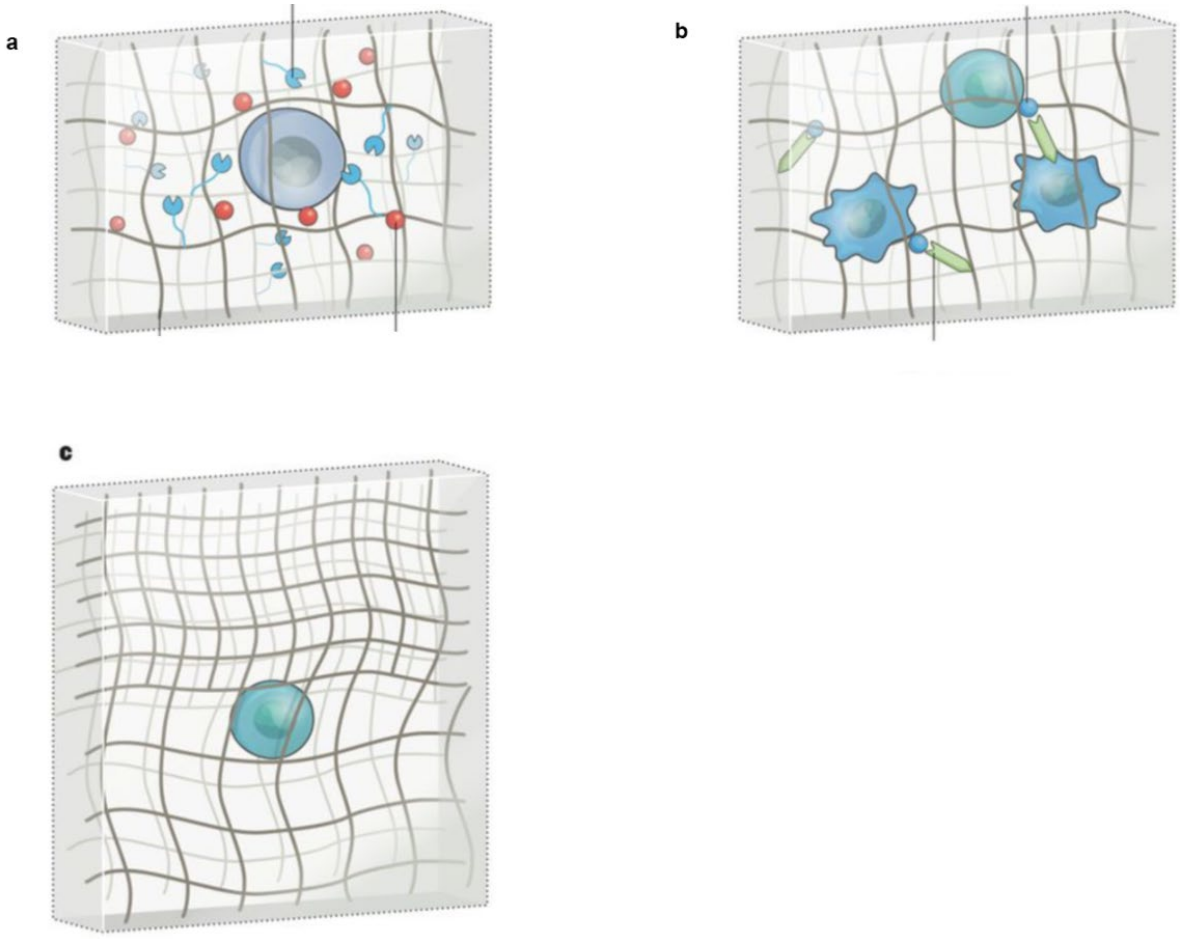
2. The artificial pancreas presented in class uses the biopolymer alginate.

    a) Alginate crosslinks in the presence of calcium ions. What other ions could be used to crosslink alginate?
    b) The crosslinking of alginate is reversible, i.e by removing calcium ions (using a calcium chelator), the gel turns into a liquid again. Is this a good thing?
    c) Alginate is a natural polymer, but unlike collagen, it is derived from marine seaweed. Explain how this could be an advantage for biostability of the implant inside the human body.
    d) Alginate is usually used to encapsulate beta islets in microcapsules. What is the advantage of using beta islets in the form of a sheet instead?
    e) How could you make alginate into sheet?

a) Looking at the period table, elements in the same column as calcium include strontium, barium, magnesium (which form divalent cations in solution) and all can be used to form gels from alginate. The heavier elements form stronger gels.
b) In vivo, it is not a good thing if the gel starts to de-polymerize, since the donor cells would then be exposed to the immune cells of the host. That is why a lot of encapsulation is being done now with barium ions which give much stronger and more stable gels. For in vitro assays, the ability to take cells easily out of the gel for analysis, has made alginate a very popular material to work with.
c) Mammalian cells produce enzymes which degrade mammalian protein, i.e. collagenase degrades collagen. Since mammalian cells will not have enzymes to degrade marine polymers, these polymers will be much more stable in the body
d) A sheet can be easily removed and replaced in one step.

e) **The easiest would be to slowly release calcium ions so the alginate could remain liquid long enough to flow and fill the bottom of the plate, before crosslinking occurs (the calcium carbonate GDL formulation). The other option would be to inject the liquid alginate into a mold, which was made of a calcium permeable material. Putting the whole mold into a calcium chloride bath would then cause the liquid to become solid in the shape of the mold.**

3. In this figure from Green and Elisseeff published in Nature, 386:540, 2016, three strategies of making a tissue engineered scaffold are illustrated. Describe the key feature of each scaffold.



From the paper: "a, Cell-matrix mimics. Synthetic hydrogels made of polymers can be modified with peptides or proteins (such as growth factors) and cell-sensitive degradable crosslinks that mimic many of the properties of the native tissue extracellular matrix (ECM)[1]. b, Cells often live in communities, so it can be useful to mimic cells by attaching surface proteins to a hydrogel[6]. c, The mechanical properties of the hydrogel can be controlled by varying the crosslinking density or using chemistries that change the mechanical properties independently of ligand

presentation; by metal ligand chemistry from the mussel[68]; or by varying the ionic crosslinking density in alginate hydrogels derived from seaweed[22].”

4. Besides pore size, the interconnectivity of the pores is an important feature of a scaffold. Discuss what determines the pore size in the below techniques and if the pores are interconnected.

    a) Porogen leaching

    b) Reverse opal templating

    c) Cyrogelation

a) Pore size determined by size of porogen (i.e. size of salt crystal). Pores can be connected with salt content is high.
b) Pore size determined by diameter of microgel (i.e. gelatin sphere). Pore are connected if one anneals the microgels together (by temperature if using gelatin).
c) Pore size is determined by the size of the ice crystals. Generally the pores are connected.