Assignment 12: Tissue Engineered Products

Cell and Tissue Engineering

Problems

1. Please answer the case study questions (a), (b) and (c) on page 436-437 in your textbook, Tissue Engineering by Saltzman.

    a. Describe the differences between hyaline cartilage and fibrocartilage. Histologically, what do these two types of tissue look like (that is, describe the cellular and extracellular features of each)?

Differences between hyaline cartilage and fibrocartilage

	Hyaline cartilage	Fibrocartilage
Color	White	Glass-like, translucent, bluish-white color
Location	Present at the connection between the ribs and the sternum, in the trachea and on the articulating surfaces of the synovial joints (such as elbow and knee)	Occurs between vertebral bodies, in the pubic symphysis, menisci, the annulus fibrosis of the intervertebral discs, and at the tendon-bone interface.
Function	Provides smoothness and lubrication of the bones at the joints.	Attaches bones to other bones and provides restricted mobility to the joints.
Extracellular Features	Matrix rich in ground substance: glycosaminoglycans (CAGs) and collagen fibers (collagen II) in a fewer number compared to fibrocartilage, making it weaker. Contains large chondrocytes in lacunae.	Matrix rich in densely braided collagen fibers (collagen I and II) making the tissue highly resistant to compression. Contains fibroblasts, fibrocytes, chondroblasts and a few chondrocytes in lacunae. The ground substance contains equal amounts of dermatan and chondroitin sulfate.
Perichondrium	Present (except the hyaline cartilage at the end of the growing bones).	Absent

Hyaline cartilage is very uniform in appearance. It is surrounded by the perichondrium. It is mainly made of chondrocytes with one or two nuclei and clear protoplasm. Chondrocytes are found in the lacunae.

Fibrocartilage is a densely arranged, whitish, fibrous tissue with a mixture of both chondrocytes and fibroblasts. It contains large bundles of collagen fibers running linearly through the tissue.

b. What features of the local site of repair would you expect to influence the outcome towards either of these endpoints?

Carticel is cultured autologous chondrocytes injected at the local site of repair. Chondrocytes synthesize components such as collagen, glycoproteins, proteoglycans and hyaluronan. They are also mainly responsible for the production of the extracellular matrix that leads to hyaline cartilage. Hyaline cartilage results from the combination of type II collagen and proteoglycans present in a specific composition and organization (Armiento et al.).
Fibrocartilage can develop at sites where entheses are subject to both shear and compressive. They are present when chondrocytes express a higher level of type I collagen down-regulating the expression of type II collagen.

c. Are there any elements that you could add to the cell suspension that is injected into the defect site, or to the surgical procedure used to deploy the cells, to increase the probability of the most desirable outcome?

Chondrocytes are altered by growth factors such as TGF-beta which stimulates the production of new cells and chondrogenesis. The effects of BMP-2 are similar to that of TGF-beta1 with increased ECM production and decreased expression of collagen type 1. Chondrogenic differentiation is enhanced when IGF-1 and TGF-beta1 are used in combination. Other growth factors have been identified to be important during cartilage repair (VEGF, BMP, FGF, Wnt) (Fortier et al.).

In addition, chemical, and mechanical cues like specific microgrooves patterns or other topological features of a 3D porous scaffold can influence the aggregation of chondrocytes increasing the success of the cartilage repair (Nikkhah et al.).

2. The review article in your assigned reading this week was co-authored by our guest lecturer Dr. Yusuf Khan. It discusses the state-of-the-art in bone tissue engineering solutions as well as the future directions for this field. After watching lecture 2 and reading this article please provide a summary of bone tissue engineering approaches, tools, limitations and prospects. (350 words or less)

AT THE END OF THE SUMMARY provide a paragraph comparing and contrasting approaches in bone tissue engineering to approaches in engineered skin grafts (300 words or less).

Autograft bone harvested from the iliac crest is the standard in bone grafts, it satisfies all the criteria of successful grafts: osteoconductivity, osteogenicity and osteoinductivity. However, it has its own limitations such as a limited supply of donor bone, and it is associated with donor-site morbidity. The other alternative is to take tissues from donors or cadavers, allograft, which addresses these two issues. Since allografts require sterilization, much of the osteoinductivity and osteogenicity are removed. However, they have their own drawbacks: reduced functional capacity, non-unions and poor-bone allograft incorporation.
Currently existing commercial bone graft products can be classified into five major categories
Allograft-based: demineralized bone matrix (DBM) is prepared using a demineralizing agent leaving the bone matrix with desired osteoinductive growth factors. DBM could be combined with bone chips, glycerol or collagen in an osteoconductive and osteoinductive scaffold.
Factor-based substitutes: growth factors residing in the ECM of the bone involved in bone regeneration; TGF-beta, IGF, PDGF, FG, VEGF and BMPs, are mixed with an autograft. In conjunction of growth factors, small molecules like statin; which increases not only bone density, but also expression of VEGF and BMP-2; or cAMP; which enhances the collagen matrix; can also be injected.
Cell-based substitutes: in vitro stem cells have been differentiated toward the osteoblast lineage. However, interaction between stem cells and scaffold is still actively investigated.
Ceramic-based substitutes: the primary component of bone is calcium phosphate making it desirable to use for graft substitutes. Likewise bioactive glass, another ceramic, can be used as composite to form bone cement or due to its mineralizing capabilities, can be combined to form a porous cartilage-bone interface.
Natural/synthetic, degradable/nondegradable polymer-based substitutes: degradable synthetic polymers include polylactic acid and poly(lactic-co-glycolic acid) (PLAGA).
By combining polymer (PLAGA) and ceramic (CaP), investigators obtain a biodegradable, formable, osteoconductive, and osteointegrative material. Polyphosphazenes (PPHOS), have also great potential for graft substitute.
Recently a novel sintered polymeric microspheres scaffold with calcium phosphate synthesized within the microsphere (Laurencin et al.), has provided a surface for osteoblast attachment, proliferation, differentiation and migration. The addition of a nanofiber has increased production of osteoblast proteins, and pre-seeded osteoblasts within the scaffold contributed to overall bone repair.

Similar to bone graft, treatment of skin wounds includes the use of autografts, allografts (usually taken from cadavers), or xenografts. Autografts like in bone graft is

the gold standard due to its low risk of rejection. Dermal skin substitutes are often made of biomaterials without any incorporated cell (acellular grafts), these scaffolds act primarily as a conduct for fibroblasts and endothelial cell migration. Cellular skin substitutes are produced using fibroblasts (dermal grafts), keratinocytes (epidermal grafts), or both (dermo-epidermal grafts). Like bone grafts, skin scaffolds are made of either natural polymers: collagen, gelatin, chitosan, fibrin, glycosaminoglycans–GAGs, and HA; or synthetic polymers: PEG, PLGA, PLACL. Like bone composites, synthetic polymers can be combined with natural polymers to produce bio-functional composite materials with desired mechanical properties and high biocompatibility. Chronic wounds are characterized by the presence of persistent infections. Artificial skin grafts have been made of polymer matrix reinforced with antimicrobial nanoparticle. Vascularization is critical in skin graft (and bone repair (Mercado-Pagán et al.)) to supply oxygen, promote healing, prevent infection, and sloughing of the implant and sepsis. Adjustments to the scaffold pore size, shape and interconnectivity are made to mediate their angiogenic tissue response, and to influence cell differentiation and propagation. Angiogenic growth factors could be incorporated to improve angiogenesis such as PDGF, VEGF or fibroblast growth factor (bFGF). HUVECS combined with fibroblasts, keratinocytes in a collagen matrix, in-vitro developed into a vascular network (similar result was obtained with HUVECS + collagen I + fibronectin + Bcl-2). Using the same design in bone tissue engineering, a PLGA and alginate-based microsphere scaffold in tissue-engineered skin, significantly improved angiogenic response when loaded with GFs (Black, A F et al). In the long term, 3D bio-printing which allows for patient-customized and on-demand of skin substitutes with either incorporated autologous or allogeneic stem cells, could play an important role in skin tissue engineering.

Reference:

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- Black, A F et al. "In vitro reconstruction of a human capillary-like network in a tissue-engineered skin equivalent." *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* vol. 12,13 (1998): 1331-40. doi:10.1096/fasebj.12.13.1331
- Fortier, Lisa A., et al. "The Role of Growth Factors in Cartilage Repair." *Clinical Orthopaedics and Related Research*, vol. 469, no. 10, Oct. 2011, pp. 2706–15. *PubMed Central*, https://doi.org/10.1007/s11999-011-1857-3.
- Laurencin, Cato, et al. "Bone Graft Substitutes." *Expert Review of Medical Devices*, vol. 3, no. 1, Jan. 2006, pp. 49–57. *DOI.org (Crossref)*, https://doi.org/10.1586/17434440.3.1.49.
- Mercado-Pagán, Ángel E., et al. "Vascularization in Bone Tissue Engineering Constructs." *Annals of Biomedical Engineering*, vol. 43, no. 3, Mar. 2015, pp. 718–29. *PubMed Central*, https://doi.org/10.1007/s10439-015-1253-3.

- Nikkhah, Mehdi, et al. "Engineering Microscale Topographies to Control the Cell–Substrate Interface." *Biomaterials*, vol. 33, no. 21, July 2012, pp. 5230–46. *DOI.org (Crossref)*, https://doi.org/10.1016/j.biomaterials.2012.03.079.

Rubric

Question	Component	Total Point Value
1	A	5
	B	5
	C	5
2	Summary	7
3	Comparison	8
Total		**30**