Mini project

Course outline: Regenerative medicine for musculoskeletal tissues

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Table of contents

1. Introduction

Musculoskeletal (MSK) conditions include injury or degeneration of muscles, bones, cartilage, tendons, ligaments, spine, joints, and nerves. According to a 2019 estimate by the WHO, 1.71 billion people worldwide suffer from musculoskeletal conditions, making it the leading cause of disability. With the aging and increasingly obese population, this number and the associated socioeconomic burden is expected to increase. The most common conditions are lower back pain, osteoarthritis and rheumatoid arthritis and neck pain. The commonly used treatments for chronic musculoskeletal conditions include pharmacological drugs which prioritize pain relief. However, this does not address the underlying molecular basis of the pathology, and therefore most conventional treatments are not disease modifying or pro-regenerative. There is a shortage of novel pro-regenerative treatments. In fact, less than 8% of the ongoing interventional trials of musculoskeletal conditions in the US involve biologic therapies such as platelet-rich-plasma, stem cells, growth factors, which may be disease modifying (Ajalik et al., 2022).

This 3 EC course is entitled 'Regenerative Medicine for Musculoskeletal Diseases'. The focus will be on four tissue types, namely, bone, cartilage, intra-vertebral disc (IVD) and meniscus. Regenerative medicine associated with tendons, ligaments and skeletal muscle tissues are not addressed in this course due to practical considerations. The research at Utrecht University and the UMCU focusses on bone, cartilage, meniscus and IVD, therefore the expertise to teach modules on other musculoskeletal tissues are currently unavailable in-house. The target audience for this course is Masters students with a biotechnology, biochemistry or biomedical background, including, but not limited to Regenerative Medicine and Technology, Biofabrication, Biology of Disease, and Cancer, Stem Cells and Developmental Biology. Students are exposed to basic concepts in musculoskeletal regeneration and tissue engineering through lectures, self-study and collaborative group projects. The course duration is 9 weeks, with a workload of 8 hours per week.

This course document details course objectives, learning resources for self-study, assessment types, involvement of local teachers, summary of tissue engineering approaches for each tissue, and next steps for setting up this course. The attached excel file contains the same information and in addition the course design. The learning objectives have been devised according to Bloom's Taxonomy, which categorizes six levels of learning, namely, knowledge (L1), comprehension (L2), application (L3), analysis (L4), synthesis (L5), and evaluation (L6). Two methods of course design i.e. organization of the Learning Units (LUs) of the course are proposed. In the first course design, the content is ordered according to the type of regenerative treatment, while in the second course design, the content is ordered according to the tissue type.

Course design 1 LUs:

1. Introduction to musculoskeletal anatomy, development and diseases
2. Cell therapy and conditioned media for MSK pathologies (bone, cartilage, meniscus, IVD)
3. Extracellular vesicles (EVs) for MSK pathologies
4. Gene therapy for MSK pathologies
5. Tissue engineering (TE) for MSK pathologies, including the TE triad: cells, biomaterials and biochemical/biomechanical stimulation
6. *In vitro* models for disease modelling
7. Clinical translation and ethics

Course design 2 LUs:

1. Introduction to musculoskeletal anatomy, development and diseases

2. Bone
a. Cell therapy
b. EVs
c. gene therapy
d. tissue engineering
e. *in vitro* models

3. Cartilage
a. Cell therapy
b. EVs
c. gene therapy
d. tissue engineering
e. *in vitro* models

4. IVD
a. Cell therapy
b. EVs
c. gene therapy
d. tissue engineering
e. *in vitro* models

5. Meniscus
a. Cell therapy
b. EVs
c. gene therapy
d. tissue engineering
e. *in vitro* models

6. Clinical translation and ethics

2. Course design 1 learning objectives

LU 1: Introduction to musculoskeletal anatomy, development and diseases
At the end of this learning unit, you can **summarize (L6)** the physiological and pathophysiological microenvironment of bone, cartilage, meniscus and IVD, and **critique (L6)** the current standard of care for common diseases of MSK tissues.

1. **Describe (L2)** tissue anatomy and composition, detailing the macroscopic appearance, cell population(s) and ECM constituents, and arrangement of these constituents.
2. **Summarize (L6)** the physiological microenvironment of the tissue, including vasculature, innervation, and oxygen tension.
3. **Analyze (L4)** biomechanical function as it relates to ECM components and organization.
4. **Describe (L2)** the basic process of tissue embryogenesis.
5. **Identify (L1)** key cells, signalling factors, pathways, and genes in tissue development.
6. **Illustrate (L3)** the four phases of the wound healing process after injury or insult (homeostasis, inflammation, proliferation, and remodelling).
7. **Name (L1)** the diseases affecting the tissue.
8. **Describe (L2)** the pathological microenvironment of the tissue as it relates to inflammation, abnormal biomechanics, imbalance of anabolism and catabolism, and cell senescence.
9. **Describe (L2)** the current standard of care. **Compare and contrast (L4)** surgical and non-surgical treatments (non-pharmacological, pharmacological, and interventional).

10. **Critique (L6)** the limitations of the current standard of care treatments in halting/ reversing degeneration and promoting regeneration.

11. **List (L1)** the clinically used treatments that are considered 'pro-regenerative' including platelet rich plasma (PRP), viscosupplementation and prolotherapy. **Judge (L6)** their advantages and disadvantages compared to the current standard of care.

LU 2: Cell therapy and conditioned media for MSK pathologies

At the end of this learning unit, you can **defend (L6)** the use of MSCs for MSK diseases, **summarize (L6)** the mechanism of its therapeutic effect, and **compare and contrast (L4)** the use of cell therapies and conditioned media for MSK pathologies.

1. **Describe (L2)** an MSC. **List (L1)** the criteria a cell must fulfil to be considered an MSC according to the International Society for Cellular Therapy.

2. **Explain (L2)** which tissues MSCs can be harvested from.

3. **List (L1)** the molecules that the MSC secretome contains, **categorize (L4)** their mechanism of action and **compare and contrast (L4)** the properties and applicability of conditioned media over cell therapy.

4. **Summarize (L6)** the therapeutic mechanism of MSCs in treating MSK pathologies and **list (L1)** cellular regulatory pathways are involved.

5. **Explain (L2)** the role tissue specific and non-tissue specific factors (eg: in vitro culture, cell seeding density, donor characteristics) and **describe (L2)** how they affect MSC phenotype and function.

LU 3: Extracellular vesicles for MSK pathologies

At the end of this learning unit, you can **summarize (L6)** the mechanism behind the pro-regenerative effect of EVs.

1. **Compare and contrast (L4)** MSC EVs and MSC cell therapy in treating MSK pathologies.

2. **Sketch (L4)** the EV composition and biogenesis; and **categorize (L5)** the EV as an apoptotic body, microvesicle or exosome based on its size and route of biogenesis.

3. **Describe (L2)** how EVs elicit cellular responses.

4. **Use (L3)** the databases Vesiclepedia and ExoCarte, to find out the protein, lipid and nucleic acid content of EVs from various sources.

5. **List (L1)** 5 ways to purify EVs.

6. Read recent state of the art pre-clinical studies about EVs and **defend (L6)** their choice of:

a. Cell source

b. EV isolation protocol

c. Administration and dosage

d. Cargo contained in the EV that has a pro-regenerative effect (eg: growth factors or miRNA)

e. Pro-regenerative effect and the underlying mechanism (classify the mechanism as matrix remodelling, angiogenesis, cell proliferation, cell polarization, cell differentiation, tissue homeostasis)

f. Animal and injury model for OA (collagenase induced knee OA or DMM or osteochondral defect), RA and fracture healing

7. **Recognize (L2)** the translational state of EV therapy (pre-clinical state, specifically small animals)

LU 4: Gene therapy for MSK pathologies

At the end of this learning unit, you can **justify (L6)** the choice of transgene(s), choice of vector and choice of *in vivo* or *ex vivo* delivery in each gene therapy study.

1. **Compare and contrast (L4)** *in vivo* and *ex vivo* gene therapy, with a focus on choosing the appropriate delivery mode based on tissue matrix composition.

2. **Select (L1)** and **justify (L6)** the commonly used transgenes i.e. genes of interest for each tissue type.

3. **Identify (L1)** commonly used viral and non-viral vectors and **describe (L2)** two advantages and disadvantages of each

4. For *ex-vivo* gene therapy, **list (L1)** commonly used cell types and **analyze (L4)** the rationale behind their use.

5. **Illustrate (L3)** the use of a gene activated matrix for *ex vivo* and *in vivo* gene therapy.

6. **Recognize (L2)** the translational state of gene therapy for use in MSK diseases (small scale trials exist, yet none have been approved by the FDA)

LU 5: Tissue Engineering for MSK pathologies

At the end of this learning unit, you can **assess (L6)** the concerted effect between cells, scaffolds, biofabrication technique and biomechanical/ biomechanical stimuli in promoting regeneration, **judge (L6)** the choice of techniques for analyzing the neo tissue formed and **defend (L6)** the relevance of the animal and injury model used.

The main tools to engineer tissues are cells, scaffolds, biochemical and biomechanical stimuli.

1. **Name (L1)** all the relevant cell sources that show promise in the repair of a particular tissue.

2. **Justify (L6)** cell choice, **comparing and contrasting (L4)** allogenic and autologous cells, as well as mature and stem/ progenitor cells in their safety and efficacy.

3. **Describe (L2)** one advantage and disadvantage of using scaffolds and scaffold-free approaches

4. **Defend (L6)** the ultimate goal of TE to recapitulate zonal and anisotropic tissue organization.

5. **List (L1)** the biomaterials used for fabricating TE scaffolds and **compare and contrast (L4)** between natural, synthetic and hybrid biomaterials

6. **Justify (L6)** the choice of a scaffold material for a specific musculoskeletal tissue - thinking about the interplay between biocompatibility, biodegradability, pore size and porosity, and mechanical properties.

7. **List (L1)** the fabrication types used for fabricating TE scaffolds- **categorize (L4)** the type of fabrication technology used.

8. **Explain (L2)** the basic principle behind commonly used musculoskeletal biofabrication approaches: extrusion-based, inkjet-based and laser-assisted printing technologies

9. For the commonly used additive manufacturing techniques, **evaluate (L6)** the most compatible bioink for that printing modality, cell viability post printing, speed and resolution (x-y plane and z axis).

10. **Demonstrate (L3)** the link between scaffold biophysical parameters (architecture, topography and mechanical properties) and hierarchical neo-tissue formation.

11. **Outline (L1)** commonly used biochemical stimuli i.e. growth factors, cytokines and oxygen tension.

12. **Summarize (L2)** the advantages of using a bioreactor for tissue maturation *in vitro*- in terms of providing the biomechanical cues and culture conditions.

13. **Assess (L6)** the most employed loading regimens and **defend (L6)** it based on physiological loading conditions experienced by a tissue.

14. **Illustrate (L4)** techniques are used to implant/ fix the bioengineered construct into the defect and analyze (L4) if it promotes integration to host tissue?

15. **Justify (L6)** analysis techniques used to characterize the properties of the neo-tissue formed (Biological properties, mechanical properties, structural properties- organization of ECM components).

16. **List (L1)** small and large animal models are used for TE, **judge (L6)** most representative animal in terms of anatomy and loading conditions. **Assess (L6)** the relevance of the acute or chronic injury model used.

17. **Predict (L4)** the challenges in engineering in large (vascularization and innervation) and multi-tissue (soft to hard tissue interface regeneration) constructs.

18. **Compare and contrast (L4)** two methods of vascularization of TE grafts: angiogenesis and iosculation.

LU 6: In vitro models for disease modelling
At the end of this learning unit, you can **compare and contrast (L4)** different disease models and **justify (L6)** the choice of OOC device, cell types, and biochemical/ biomechanical stimuli for applications in basic science and disease modelling.

1. **Name (L1)** the *in vivo*, *ex vivo*, and *in vitro* models of MSK tissues (animal models, tissue explants, macroscale bioreactors, 2D cell culture, 3D biomaterial-based culture models, organoids, and organ-on-a-chip)

2. **Compare and contrast (L4)** the advantages and disadvantages of these models in disease modelling, and drug testing.

3. **List (L1)** the components and **describe (L2)** the principles behind tissue chips.

4. For each tissue OOC, **justify (L6)** the choice of device characteristics, cell types, biochemical/ biomechanical stimuli.

5. **Explain (L2)** the advantages and limitations of a specific OOC model for basic science/ disease modelling/ drug testing.

LU 7: Clinical translation and ethics
At the end of this learning unit, you can **summarize (L6)** challenges for translating various regenerative therapeutics from bench to bedside, **interpreting (L6)** the regulatory, manufacturing, standardization, and intellectual property hurdles.

1. For each regenerative therapy, **identify (L1)** which regulatory framework it is governed by i.e. Advanced Therapy Medicinal Product (ATMP) or pharmaceutical product.

2. **Explain (L2)** what an ATMP is and **describe (L2)** the 4 main categories of ATMPs?

3. **Analyze (L4)** the barriers to the translation of an ATMP product, focusing on standardization and quality control.

4. **Summarize (L6)** ethical issues arise at the various stages of regenerative medicine research (laboratory, pre-clinical and human trial stages).

3. Learning resources for self-study

LU 1: Introduction to Musculoskeletal anatomy, development, and diseases
Hart, D. A., Nakamura, N., & Shrive, N. G. (2021). Perspective: Challenges Presented for Regeneration of Heterogeneous Musculoskeletal Tissues that Normally Develop in Unique Biomechanical Environments. *Frontiers in Bioengineering and Biotechnology*, *9*(September), 1–9. https://doi.org/10.3389/fbioe.2021.760273

Cooper, G., Herrera, J., Kirkbride, J., & Perlman, Z. (2020). Regenerative Medicine for Spine and Joint Pain. In *Regenerative Medicine for Spine and Joint Pain*. https://doi.org/10.1007/978-3-030-42771-9

Baykal, B., & Korkusuz, P. (2016). Development of the Musculoskeletal System. In *Musculoskeletal Research and Basic Science* (pp. 289–302). Springer International Publishing. https://doi.org/10.1007/978-3-319-20777-3_17

Bone development: intramembranous vs endochondral ossification (12 minutes)
https://www.youtube.com/watch?v=MnFClh08UKM

Week by week explanation of the development of the skeletal system: axial skeleton (neurocranium and visceral cranium) and thoracic cage, joints and upper limbs and lower limbs (49 minutes) https://www.youtube.com/watch?v=WmlbqVyhMts

Development of the vertebral column (15 minutes) https://www.youtube.com/watch?v=R9Q2ThMbHU8

LU 2: Cell therapy and conditioned media for MSK pathologies

Andia, I., & Maffulli, N. (2019). New biotechnologies for musculoskeletal injuries. *Surgeon*, *17*(4), 244–255. https://doi.org/10.1016/j.surge.2018.08.004

Angele, P., Docheva, D., Pattappa, G., & Zellner, J. (2022). Cell-based treatment options facilitate regeneration of cartilage, ligaments and meniscus in demanding conditions of the knee by a whole joint approach. Knee Surgery, Sports Traumatology, Arthroscopy, 30(4), 1138–1150. https://doi.org/10.1007/s00167-021-06497-9

Bian, Y., Wang, H., Zhao, X., & Weng, X. (2022). Meniscus repair: up-to-date advances in stem cell-based therapy. *Stem Cell Research & Therapy*, *13*(1), 207. https://doi.org/10.1186/s13287-022-02863-7

Binch, A. L. A., Fitzgerald, J. C., Growney, E. A., & Barry, F. (2021). Cell-based strategies for IVD repair: clinical progress and translational obstacles. *Nature Reviews Rheumatology*, *17*(3), 158–175. https://doi.org/10.1038/s41584-020-00568-w

Hulme, C. H., Perry, J., McCarthy, H. S., Wright, K. T., Snow, M., Mennan, C., & Roberts, S. (2021). Cell therapy for cartilage repair. *Emerging Topics in Life Sciences*, *5*(4), 575–589. https://doi.org/10.1042/ETLS20210015

Iaquinta, M. R., Mazzoni, E., Bononi, I., Rotondo, J. C., Mazziotta, C., Montesi, M., Sprio, S., Tampieri, A., Tognon, M., & Martini, F. (2019). Adult Stem Cells for Bone Regeneration and Repair. Frontiers in Cell and Developmental Biology, 7(November), 1–15. https://doi.org/10.3389/fcell.2019.00268

Kangari, P., Talaei-Khozani, T., Razeghian-Jahromi, I., & Razmkhah, M. (2020). Mesenchymal stem cells: amazing remedies for bone and cartilage defects. *Stem Cell Research & Therapy*, *11*(1), 492. https://doi.org/10.1186/s13287-020-02001-1

Kwon, D. G., Kim, M. K., Jeon, Y. S., Nam, Y. C., Park, J. S., & Ryu, D. J. (2022). State of the Art: The Immunomodulatory Role of MSCs for Osteoarthritis. *International Journal of Molecular Sciences*, *23*(3), 1618. https://doi.org/10.3390/ijms23031618

Lattermann, C., Leite, C. B. G., Frisbie, D. D., Schlegel, T. S., Bramlage, L. R., Koch, T., Centeno, C., Goodrich, L. R., Johnstone, B., Trumper, R., Watts, A., Little, C., Barry, F., Guilak, F., & McIlwraith, C. W. (2022). Orthobiologics in orthopedic applications: A report from the TMI Havemeyer meeting on orthobiologics. *Journal of Cartilage & Joint Preservation*, *April*, 100055. https://doi.org/10.1016/j.jcjp.2022.100055

Veronesi, F., Borsari, V., Sartori, M., Orciani, M., Mattioli-Belmonte, M., & Fini, M. (2018). The use of cell conditioned medium for musculoskeletal tissue regeneration. *Journal of Cellular Physiology*, *233*(6), 4423–4442. https://doi.org/10.1002/jcp.26291

LU 3: Extracellular vesicles for MSK pathologies

Abreu, H., Canciani, E., Raineri, D., Cappellano, G., Rimondini, L., & Chiocchetti, A. (2022). Extracellular vesicles in musculoskeletal regeneration: Modulating the therapy of the future. *Cells*, *11*(1), 1–19. https://doi.org/10.3390/cells11010043

Herrmann, M., Diederichs, S., Melnik, S., Riegger, J., Trivanović, D., Li, S., Jenei-Lanzl, Z., Brenner, R. E., Huber-Lang, M., Zaucke, F., Schildberg, F. A., & Grässel, S. (2021). Extracellular Vesicles in Musculoskeletal Pathologies and Regeneration. *Frontiers in Bioengineering and Biotechnology*, *8*(January). https://doi.org/10.3389/fbioe.2020.624096

Malda, J., Boere, J., Van De Lest, C. H. A., Van Weeren, P. R., & Wauben, M. H. M. (2016). Extracellular vesicles - New tool for joint repair and regeneration. *Nature Reviews Rheumatology*, *12*(4), 243–249. https://doi.org/10.1038/nrrheum.2015.170

Yao, X., Wei, W., Wang, X., Chenglin, L., Björklund, M., & Ouyang, H. (2019). Stem cell derived exosomes: microRNA therapy for age-related musculoskeletal disorders. *Biomaterials*, *224*(May), 119492. https://doi.org/10.1016/j.biomaterials.2019.119492

LU 4: Gene therapy for MSK pathologies

Andia, I., & Maffulli, N. (2019). New biotechnologies for musculoskeletal injuries. *Surgeon*, *17*(4), 244–255. https://doi.org/10.1016/j.surge.2018.08.004

Evans, C. H., & Huard, J. (2015). Gene therapy approaches to regenerating the musculoskeletal system. *Nature Reviews Rheumatology*, *11*(4), 234–242.

Madrigal, J. L., Stilhano, R., & Silva, E. A. (2017). Biomaterial-guided gene delivery for musculoskeletal tissue repair. *Tissue Engineering - Part B: Reviews*, *23*(4), 347–361. https://doi.org/10.1089/ten.teb.2016.0462

Watson-Levings, R. S., Palmer, G. D., Levings, P. P., Dacanay, E. A., Evans, C. H., & Ghivizzani, S. C. (2022). Gene Therapy in Orthopaedics: Progress and Challenges in Pre-Clinical Development and Translation. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.901317

LU 5: Tissue Engineering for MSK pathologies

Kwon, H., Brown, W. E., Lee, C. A., Wang, D., Paschos, N., Hu, J. C., & Athanasiou, K. A. (2019). Surgical and tissue engineering strategies for articular cartilage and meniscus repair. *Nature Reviews Rheumatology*, *15*(9), 550–570. https://doi.org/10.1038/s41584-019-0255-1

Gkantsinikoudis, N., Kapetanakis, S., Magras, I., Tsiridis, E., & Kritis, A. (2022). Tissue Engineering of Human Intervertebral Disc: A Concise Review. *Tissue Engineering Part B: Reviews*, *28*(4). https://doi.org/10.1089/ten.teb.2021.0090

Bilgen, B., Jayasuriya, C. T., & Owens, B. D. (2018). Current Concepts in Meniscus Tissue Engineering and Repair. *Advanced Healthcare Materials*, *7*(11), 1–13. https://doi.org/10.1002/adhm.201701407

Calejo, I., Costa-Almeida, R., Reis, R. L., & Gomes, M. E. (2020). A Physiology-Inspired Multifactorial Toolbox in Soft-to-Hard Musculoskeletal Interface Tissue Engineering. *Trends in Biotechnology*, *38*(1), 83–98. https://doi.org/10.1016/j.tibtech.2019.06.003

Dou, Y., Sun, X., Ma, X., Zhao, X., & Yang, Q. (2021). Intervertebral Disk Degeneration: The Microenvironment and Tissue Engineering Strategies. *Frontiers in Bioengineering and Biotechnology*, *9*(July), 1–18. https://doi.org/10.3389/fbioe.2021.592118

Hart, D. A., Nakamura, N., & Shrive, N. G. (2021). Perspective: Challenges Presented for Regeneration of Heterogeneous Musculoskeletal Tissues that Normally Develop in Unique Biomechanical Environments. *Frontiers in Bioengineering and Biotechnology*, *9*(September), 1–9. https://doi.org/10.3389/fbioe.2021.760273

Li, J., Kim, C., Pan, C. C., Babian, A., Lui, E., Young, J. L., Moeinzadeh, S., Kim, S., & Yang, Y. P. (2022). Hybprinting for musculoskeletal tissue engineering. *IScience*, *25*(5), 104229. https://doi.org/10.1016/j.isci.2022.104229

Potyondy, T., Uquillas, J. A., Tebon, P. J., Byambaa, B., Hasan, A., Tavafoghi, M., Mary, H., Aninwene, G. E., Pountos, I., Khademhosseini, A., & Ashammakhi, N. (2021). Recent advances in 3D bioprinting of musculoskeletal tissues. *Biofabrication*, *13*(2). https://doi.org/10.1088/1758-5090/abc8de

Zhang, X., Wang, D., Mak, K. L. K., Tuan, R. S., & Ker, D. F. E. (2021). Engineering Musculoskeletal Grafts for Multi-Tissue Unit Repair: Lessons From Developmental Biology and Wound Healing. *Frontiers in Physiology*, *12*(August). https://doi.org/10.3389/fphys.2021.691954

Xie, C., Ye, J., Liang, R., Yao, X., Wu, X., Koh, Y., Wei, W., Zhang, X., & Ouyang, H. (2021). Advanced Strategies of Biomimetic Tissue-Engineered Grafts for Bone Regeneration. *Advanced Healthcare Materials*, *10*(14), 1–18. https://doi.org/10.1002/adhm.202100408

LU 6: In vitro models for disease modeling

Ajalik, R. E., Alenchery, R. G., Cognetti, J. S., Zhang, V. Z., McGrath, J. L., Miller, B. L., & Awad, H. A. (2022). Human Organ-on-a-Chip Microphysiological Systems to Model Musculoskeletal Pathologies and Accelerate Therapeutic Discovery. *Frontiers in Bioengineering and Biotechnology*, *10*(March), 1–23. https://doi.org/10.3389/fbioe.2022.846230

Arrigoni, C., Lopa, S., Candrian, C., & Moretti, M. (2020). Organs-on-a-chip as model systems for multifactorial musculoskeletal diseases. *Current Opinion in Biotechnology*, *63*, 79–88. https://doi.org/10.1016/j.copbio.2019.12.006

Banh, L., Cheung, K. K., Chan, M. W. Y., Young, E. W. K., & Viswanathan, S. (2022). Advances in organ-on-a-chip systems for modelling joint tissue and osteoarthritic diseases. *Osteoarthritis and Cartilage*, *30*(8), 1050–1061. https://doi.org/10.1016/j.joca.2022.03.012

Kahraman, E., Ribeiro, R., Lamghari, M., & Neto, E. (2022). Cutting-Edge Technologies for Inflamed Joints on Chip: How Close Are We? *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.802440

Mainardi, A., Cambria, E., Occhetta, P., Martin, I., Barbero, A., Schären, S., Mehrkens, A., & Krupkova, O. (2022). Intervertebral Disc-on-a-Chip as Advanced In Vitro Model for Mechanobiology Research and Drug Testing: A Review and Perspective. *Frontiers in Bioengineering and Biotechnology*, *9*(January), 1–18. https://doi.org/10.3389/fbioe.2021.826867

Paggi, C. A., Teixeira, L. M., Le Gac, S., & Karperien, M. (2022). Joint-on-chip platforms: entering a new era of in vitro models for arthritis. Nature Reviews Rheumatology, 18(4), 217–231. https://doi.org/10.1038/s41584-021-00736-6

LU 7: Clinical translation and ethics

Lattermann, C., Leite, C. B. G., Frisbie, D. D., Schlegel, T. S., Bramlage, L. R., Koch, T., Centeno, C., Goodrich, L. R., Johnstone, B., Trumper, R., Watts, A., Little, C., Barry, F., Guilak, F., & McIlwraith, C. W. (2022). Orthobiologics in orthopedic applications: A report from the TMI Havemeyer meeting on orthobiologics. *Journal of Cartilage & Joint Preservation*, *April*, 100055. https://doi.org/10.1016/j.jcjp.2022.100055

Baker, H. B., McQuilling, J. P., & King, N. M. P. (2016). Ethical considerations in tissue engineering research: Case studies in translation. *Methods*, *99*(2016), 135–144. https://doi.org/10.1016/j.ymeth.2015.08.010

Zuncheddu, D., Della Bella, E., Schwab, A., Petta, D., Rocchitta, G., Generelli, S., Kurth, F., Parrilli, A., Verrier, S., Rau, J. V., Fosca, M., Maioli, M., Serra, P. A., Alini, M., Redl, H., Grad, S., & Basoli, V. (2021). Quality control methods in musculoskeletal tissue engineering: from imaging to biosensors. *Bone Research*, *9*(1). https://doi.org/10.1038/s41413-021-00167-9

4. Types of assessment

After every learning unit (LU), students prepare 3 multiple choice questions, 3 glossary terms and their definitions, and a summary (200 words approximately) of 1 important concept in the LU. Ideally, students identify the important concepts and divide them up amongst themselves to avoid overlap. These are verified for quality by the course instructor before being added into a google document, thereby the google document provides a summary of every LU in the course. The submission of these components and consequently active participation in the course counts for 10% of the overall grade.

Since this is the first time this course is being offered, student feedback is critical to improve the course. Weekly forms or forms after the completion of each learning unit can be provided to students, the filling of which could account for 10% of the final grade. It should not take more than 10 minutes. A few possible feedback questions are listed below.

Question	Possible answer set
The sequence of concepts was effectively organized and helped in understanding this module.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
The teaching activities were appropriate for this module i.e. lectures and interactive modules. Please elaborate.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree Open ended
Which concepts were difficult to understand, and why?	Open ended
The self-study materials, including review articles and textbook chapters, aided my understanding of the module content.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
How many hours did you spend on self-study, working on assessments or any other course related work in this module (apart from lectures)?	Open ended

Below, various possible assessment types are presented, which will represent a major portion of the overall grade. Either a single assessment type can be selected to make up **80% of the overall grade** or two assessment points can be selected, with **each making up 40%** of the overall grade respectively.

1. Poster presentation of a landmark study (pre-clinical or clinical) about regenerative therapies or tissue engineered products for MSK tissues. The key findings, larger context and limitations of the study are presented in the form a poster (A0) presentation in pairs.

2. Graphical abstract of a landmark study
Increasingly journals have been requesting authors to submit a graphical abstract along with the body of the article. A graphical abstract is a single panel image that gives readers the take-home message of the paper. Through this assignment, students will learn to critically assess the context of a research study, the methodology employed and the main outcome of the study. In addition, students will learn to represent this knowledge in a visually appealing pictorial form. Thereby they become familiar with commonly used softwares to prepare original images for a journal article such as photoshop, illustrator, BioRender and PowerPoint.

3. Pitch presentation of a regenerative medicine start-up.
Students use the tools and concepts learnt in the course to develop a start-up idea for a regenerative therapy/ tissue engineered product to address diseases associated with either bone, cartilage, meniscus or IVD. The presentation should include the disease being addressed, the mechanism of action of the novel therapeutic product, advantages over standard of care, and plan for addressing challenges associated with GMP manufacturing, regulatory and intellectual property hurdles, and commercialization. Through this project, students will learn about the process of bench to bedside translation of novel regenerative medicine therapeutics. In addition, they will learn to develop and pitch a start-up plan.

A possible guest lecturer/ mentor for this module could be Stefan Bram, the founder and CEO of Nardia and Cellistic, which specialize in iPSC-based drug discovery and cell therapy. Other possible mentors could be among the employees at Dutch regenerative medicine companies, namely Mimetas, Necstgen and MIDA Biotech. Mimetas specializes in organ-on-a-chip models of numerous tissues for drug discovery and development. Necstgen is a company owned by the Leiden University Medical Centre that offers various types of expertise in the translation of research and early-stage clinical programs into next generation therapeutics for patients. MIDA biotech focusses on gene and stem cell therapy for age-related disorders, injuries and degenerative diseases. Lastly the employees at Utrechtinc have extensive experience with helping biomedical start-ups and as such would be valuable mentors for this module.

www.mimetas.com/en/home/
https://necstgen.com/about-us/
https://midabiotech.com/partnerships

In addition to continuous feedback questions for students to answer during the course, some feedback questions that can be provided at the end of the course (does not count for part of the course grade) are listed below:

Question	Answer set
How did you hear about this course?	Open ended
The course information was pitched at the right level. Please elaborate.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree Open ended
The instructor's teaching style was effective.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
I had enough opportunities to interact with my tutors.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
I had enough opportunities to interact with my peers.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
This course met my overall expectations.	Strongly disagree, disagree, neither agree nor disagree, agree, strongly agree
Do you have any suggestions to improve the learning experience of the course?	Open ended

5. Involvement of local teachers

Miguel Castilho: biomaterials and biofabrication of bone and cartilage
Keita Ito: cell therapies, biomaterials and biofabrication of cartilage and IVD
Riccardo Levato: cell therapies, TE, biofabrication of meniscus and cartilage
Marianna Tryfonidou: cell therapies, EVs, TE, biomaterials and biofabrication of IVD
Jos Malda: cell therapies, EVs, gene therapy, TE, biomaterials and biofabrication of cartilage
Debby Gawlitta: cell therapies, TE, biomaterials and biofabrication of bone
Saber Amin Yavari: biomaterials and biofabrication of bone
Yang Li: *in vitro* models cartilage, bone, and meniscus
Paulina Nunez: cell therapies, TE, biomaterials, biofabrication of meniscus and *in vitro* joint models
Paree Khokhani: cell therapies and EVs of bone
Frances Bach/ Josette van Maanen: EVs of IVD
Deepani Poramba-Liyanage/ Lisanne Laagland/ Xiaole Tong: cell therapies of IVD
Lizette Utomo: cell therapies, TE, and biomaterials of bone

6. Summary of tissue engineering approaches for each tissue

Meniscus
The gold standard for meniscus repair still remains a partial or total meniscectomy, which involves the removal of the damaged part of the meniscus and is performed arthroscopically. Meniscus allografts are performed almost as widely as meniscectomies. Collagen meniscus implants, which are synthetic cell-free implants, are in clinical use in the USA. In Europe, Actifit, an acellular scaffold composed of 80% PCL and 20% polyurethane has been approved for clinical use. While both these acellular scaffolds offer short term improvement of symptoms, they undergo shrinkage and shape change as well as do not promote regeneration despite cell infiltration.
In meniscus TE the main strategies for re-capitulating the zonal structure of the meniscus (red-red, red-white and white-white zone) are: scaffolds with varying porosities, controlling 3D printing

parameters, seeding different cell types at different parts of the scaffold (fibroblast-like cells in the outer zone and chondrocyte-like cells in the inner zone), and zone dependent mechanical stimulation. Common cell types used for meniscus TE are meniscal fibroblasts, MSCs (bone marrow, adipose or synovium derived) and chondrocytes co-cultured with other differentiated cells such as tenocytes, ligament fibrocytes or meniscus fibroblasts). Common biochemical stimuli are transforming growth factor (TGF- β), bone morphogenic proteins (BMP-2 and BMP-7) fibroblast growth factors (FGFs), platelet derived growth factors (PDGFs) and insulin like growth factors-1 (IGF-1). Changes in oxygen tension on the other hand have not yielded promising results.

Commonly used biomaterials are synthetic materials like polylactides and polyglycolides, as well as natural polymer such as agarose, GelMA, collagen, hyaluronic acid, decellularized ECM or combinations of these. PCL is most amenable to being processed by additive manufacturing techniques especially extrusion-based printing, where zonal architectures can be elicited through fiber spacing, fiber orientation and offset between layers. Most dominant are those 3D printed scaffolds that can replicate the circumferential and radial collagen fiber orientation of native meniscal tissue. Since the meniscus is under compression and tensile stresses in its native environment, both are important when considering loading regimens. Dual dynamic loading comprising of both tensile and compressive forces are usually employed, by either increasing both forces from the outer zone to inner zone or using tensile loading for the outer zone and compressive loading for the inner zone (Abbadessa et al., 2021; Bilgen et al., 2018; Kwon et al., 2019)

Cartilage

Currently used clinical repair strategies for cartilage defects include microfracture, osteochondral autografts, osteochondral allografts, processed allograft cartilage such as DeNovo NT, ProChondrix and Cartiform, and matrix-induced autologous chondrocyte implantation (MACI). Similar to meniscus tissue engineering, cartilage TE approaches in the recent years have moved towards replicating the anisotropic structural and biomechanical properties of cartilage.

Common cell types for cartilage regeneration are articular chondrocytes, MSCs (bone marrow, adipose, skin or synovium derived) and articular cartilage progenitor cells (ACPCs). Chondrocytes, much like meniscus fibroblasts, usually suffer from de-differentiation and loss of phenotype when expanded *in vitro,* therefore nasal or costal chondrocytes have emerged as new cell sources (Kwon et al., 2019).

Natural polymers such as hyaluronic acid, chondroitin sulphate, alginate, agarose, chitosan, gellan gum, collagen, gelatin and silk fibroin are commonly used due to their structural similarity to proteoglycans of the ECM. Gelatin is chemically methacrylated to form GelMA. Scaffolds composed of synthetic polymers such as polycaprolactone (PCL), poly (ethylene glycol) (PEG), polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic acid-co-glycolic acid) (PLGA), , poly(vinyl alcohol) (PVA), poly (propylene fumarate) (PPF), poly(L-glutamic acid), and poly(N-isopropyl acrylamide)(PNIPAAm) offer some advantages over natural polymers in terms of their ease of manufacture and control over molecular weight and degradation rate (Wei & Dai, 2021; Zhou et al., 2020). Usually chondral scaffolds are composed of hydrogels of natural or synthetic polymers to mimic the hydrated and viscoelastic cartilage ECM.

Bioreactors are commonly used for maturation of the construction by applying biophysical and biochemical stimuli. Common biochemical stimuli are transforming growth factor (TGF- β), bone morphogenic proteins (BMP-2 and BMP-7), fibroblast growth factor (FGF-2) and insulin like growth factors-1 (IGF-1) and low oxygen tension. Biomechanical stimuli include direct compression, hydrostatic pressure, shear, and tensile loading and combinations thereof. In addition to scaffold materials and cells, the architecture plays an important role in the deposition of zonal ECM, whereby studies are moving towards multiphasic or gradient architectures which have a superficial zone, middle zone, deep zone and calcified zone. The most prevalent bioprinting technology for cartilage is extrusion printing, due to its ability to deposit hydrogels in a layer-by-layer-structure. Often hydrogels

are reinforced with a fibrous mesh composed of PCL fabricated through Melt Electrowriting to improve the mechanical properties. The interaction of the fibrous mesh and hydrogels is reminiscent of the interaction of glycosaminoglycans and collagen fibrils which render cartilage its load bearing ability (Armiento et al., 2018; Salinas et al., 2018; Stampoultzis et al., 2021).

Bone

While meniscus and cartilage have very limited intrinsic repair capabilities, bone is capable of self-repair due to its vascularized and innervated nature. However critical size bone defects larger than 2.5 to 3 cm do not heal spontaneously. The current standard of care is autologous bone grafts, but allografts and xenografts are also used (Yazdanpanah et al., 2022).

Osteoblasts are tissue resident cells which are responsible for forming new bone, but there are challenges associated with harvesting, expanding, and maintaining the phenotype of these primary cells. As opposed to using fully differentiated osteoblasts, MC3T3-E1 pre-osteoblast cells can differentiate into mature osteoblasts under the right cues. As in other tissues, stem and progenitor cells and commonly used. Bone marrow mesenchymal stem cells are the most prevalent, as well as MSCs from other origins such as adipose and synovium. Dental pulp-derived stem cells (DPSCs) are also increasingly being used for bone TE (Perez et al., 2018) .

The most ubiquitously used materials in bone tissue engineering are calcium phosphates. Natural (collagen, silk, chitosan, alginate) and synthetic polymers (PCL, PGA and their co-polymers) and metals (stainless steel, titanium-alloy, cobalt–chromium-based, aluminum, lead and silver) are also employed in bone TE. The most common calcium phosphates are β-tricalcium phosphate, hydroxyapatite, bioglass, and bi-phasic calcium phosphates (BCPs). Bi-phasic calcium phosphates combine the superior solubility of β-tricalcium phosphate with the improved mechanical strength of hydroxyapatite. Bioceramics can also be combined with synthetic polymers such as PCL to stimulate biomineralization, combat the brittleness of bioceramics and better approximate the mechanical properties of the host tissue. Most common 3D printing techniques compatible with the use of the above mentioned materials are inkjet, laser-assisted and extrusion-based printing. Internal structural features of the scaffold such as pore size, porosity, pore geometry, internal geometry and fiber arrangement can also be modulated to improve structural and osteoinductive properties of the scaffold (Yazdanpanah et al., 2022).

Bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor-β1 (TGF-β1), and insulin-like growth factor 1 (IGF-1) are commonly used for bone TE. Since vascularization is a hurdle for large bone defects, VEGF can play a role in inducing angiogenesis (Perez et al., 2018). Perfusion bioreactors have been used for disseminating oxygen and nutrients through the TE graft and providing shear stress to the cells. Other mechanical stimuli are hydrostatic pressure, compression and tension, oscillatory fluid shear stress, acoustic and electromagnetic stimuli (Hao et al., 2021).

Intravertebral disc

The human IVD is a 3D cylindrical disc which connects adjacent vertebrae of the spine and thereby ensures spinal mobility and articulation of the vertebrae. Unlike the other tissues discussed so far it is not part of the knee. The current standard of care for degenerative disc disease, a major contributor to low back pain, is decompression and fusion of the spinal segments i.e. spinal fusion. However this completely destroys mobility in that specific spinal segment (Gkantsinikoudis et al., 2022).

Clinical trials for degenerative disc disease performed so far involve intradiscal injections of various drugs and biologics including autologous or allogenic MSCs, chondrocytes, allogenic MSCs + hyaluronic acid, allogenic MSCs + hyaluronic acid+ Rexlemestrocel-L, Recombinant human growth and differentiation factor-5, platelet rich plasma, corticoids and YH14618 (a drug). In addition one clinical trial explored using a prosthetic disc (Mohd Isa et al., 2022). In pre-clinical studies, cell therapy, treatment with small biologic molecules and local injection of anti-inflammatory drugs dominate as compared to tissue engineering approaches, possibly due to the unique microenvironment

of IVDs (avascular, low cell density, high osmolarity, hypoxic, high mechanical loads, and low diffusion of metabolites) (Dou et al., 2021).

To mimic the annulus fibrosis (AF), electrospinning with polymers such as polycaprolactone (PCL), PLA (poly lactic acid) and polyurethane (PU) is a common technique. Commonly used cells in pre-clinical studies are AF cells (human or animal origin) or MSCs. Electrospinning of polymer solutions yields polymer sheets which can be wound in a circle to replicate the AF structure. On the other hand, 3D printing techniques can be used for both nucleus pulposus (NP) and AF components of the IVD. Common materials are silk fibroin, PLA-GG-PEGDA, nanofiber reinforced chitosan, hyaluronic acid, and chondroitin sulphate. NP/ notochord cells/ cartilage end plate cells, as well as AF cells and MSCs are the most common cells (Pieri et al., 2020).

Common growth factors for AF and NP tissue engineering are transforming growth factor-β1 (TGF-β1 and TGF-β3), bone morphogenetic protein (BMP-2 and BMP-7), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin-like growth factor-I (IGF-I), and platelet-derived growth factor (PDGF)(Chu et al., 2018). Bioreactors have been used to apply mechanical forces that IVDs experience *in vivo* including compression, tension, bending and torsion (Šećerović et al., 2022).

7. Next steps and challenges in setting up this course

The next step in getting this course set up is gauging demand. While Debby has already sent out a form on the WhatsApp group of RMT students, there are other avenues that can be explored. For instance, a form can be sent out with the monthly LS seminars or emails to GSLS students via course coordinators, which can be used to see how many students from other GSLS masters would be interested in a course in MSK RMT. A part of gauging interest is understanding who exactly our target audience is for this course. While we envision that our target demographic is RMT and Biofabrication Masters students along with some students from other Masters such as Cancer Stem cells and Developmental Biology and Biology of Disease, the actual student composition might look different. Conversations with RMT students and alumni revealed that students who are extremely interested in MSK RMT would rather explore their interest through a major or minor internship rather than a 3EC theoretical course. In this case, our target audience might be students (from RMT/ Biofab/ other Masters) who are not fully sure if they are interested in MSK RMT and through such a course, want to explore if they are interested enough to pursue an internship or PhD in this field.

After gauging interest and ascertaining student composition, the next step is to find course instructors and simultaneously finalize learning objectives, course design and content, appropriate pedagogies, study materials and evaluation accordingly. During this process of coordinating the time availability and commitment from course instructors, the course timing (which time of the year the course will be offered) and schedule can also be decided upon. Since this is the first time this course is being offered, feedback from students is critical. This can be organized via weekly course feedback/ reflection forms, the filling of which could contribute to the final grade. See section ‘Types of assessment’ for more information. Lastly, after the course has been fully set up, it needs to be marketed via the GSLS students page/ LS seminars/ emails to GSLS students via course coordinators, to spread awareness of the possibility and logistics of undertaking this course.

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