Knee Osteoarthritis: *In Situ* Bioprinting As The Future of Joint Repair

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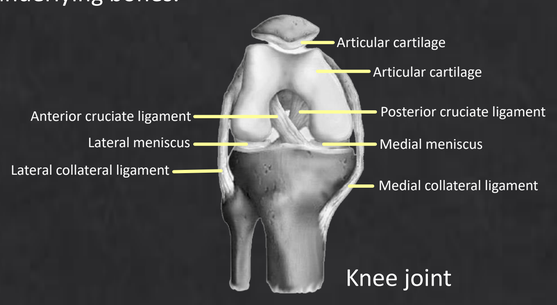
Objective: Advancements in tissue engineering have heralded the promise of *in situ* bioprinting as the future of joint repair, as current treatments are symptomatic and do not reverse the underlying pathophysiology.

1. Knee Anatomy

The knee joint is a weight-bearing synovial joint composed of three bones: femur, tibia and patella. As a weight-bearing joint, it requires several major ligaments to provide stability. These include anterior cruciate, posterior cruciate, medial collateral, and lateral collateral ligaments. Moreover, there are medial and lateral menisci which are fibrocartilaginous structures that provide stability, lubrication and nutrition to the joint space. With an increase in age and use, the intense mechanical load over many years on the knee joint can cause degenerative changes that deteriorate the joint structures surrounding the knee and cause a remodelling of the underlying bones.



Knee joint

1.2 Synovial joint

A joint is formed by the articulation of bones to permit certain movements. Synovial joints are freely moving joints that are formed by the connection between bones separated by an articular cavity. In addition to this, there are several characteristic features that also identify a synovial joint.

Firstly, the articulating surfaces of the bones involved in the joint are covered with hyaline (glass-like) articular cartilage which has viscoelastic properties due to the secretion of extracellular matrix composed of type II collagen, proteoglycans and water. This property serves as a low friction surface for bone articulation and facilitates the transmission of loads to the underlying bone. The articular cartilage is also avascular, aneural and alymphatic, therefore any wear and tear to the articular cartilage can go unnoticed and there will not be any regeneration of the cartilage lost.

A second characteristic feature of synovial joints is the joint capsule consisting of an outer fibrous capsule and an inner synovial membrane. The outer fibrous capsule completely encloses the synovial joint and provides it with stability. It is perforated by nerves and vessels that innervate and supply blood to the joint. Its inner surface is lined by synovial membrane, an important structure that secretes synovial fluid for the lubrication and nutrition of the enclosed structures such as the articular cartilage.

Other common but not universal features of synovial joints include the presence of fat pads, bursae and articular discs. These structures are in place to facilitate smooth movements and compressive forces.

2. Osteoarthritis

Osteoarthritis (OA) is the most prevalent form of joint disease characterised by intrinsic degeneration of cartilage resulting in structural and functional changes to the synovial joint. The destruction of cartilage and therefore joint damage causes the release of inflammatory mediators that further perpetuate and worsen the damage. In many instances, OA is an insidious disease that occurs progressively as an aging phenomenon. However, it is not directly caused by increasing age. Other risk factors for the development of OA include obesity, repeated stress on the joint, joint injuries and genetic predisposition.

2.2 Treatment and Management

The aims of management are:
- To educate patients about the disease
- To provide support for self-management
- To minimise pain
- To optimise function and participation in daily activities
- To intervene disease processes and its outcomes

Management of an osteoarthritic knee depends on the stage of the disorder, the severity of the symptoms, the age of the patient and the patient's functional needs. It can be split into three broad categories: (1) non-pharmacological, (2) pharmacological, and (3) surgical management.

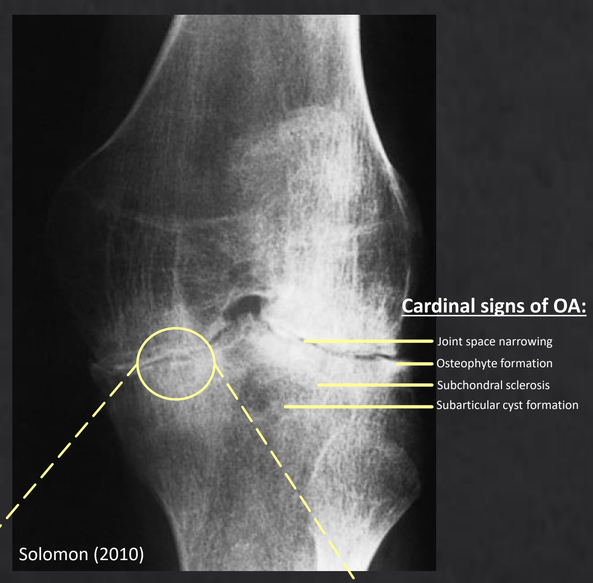
1) Non-pharmacological management includes:

2) Pharmacological management includes:

Analgesic medications
- Paracetamol
- NSAIDs
- Opioid analgesics
- Glucosamine compounds

3) Surgical management includes:

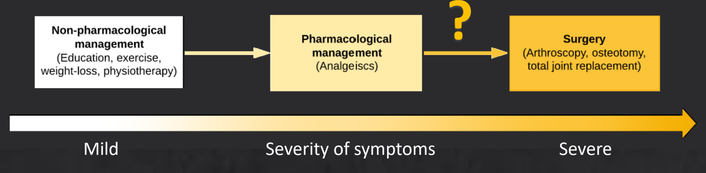
a. Arthroscopic debridement and lavage
b. Osteotomy
c. Total joint replacement

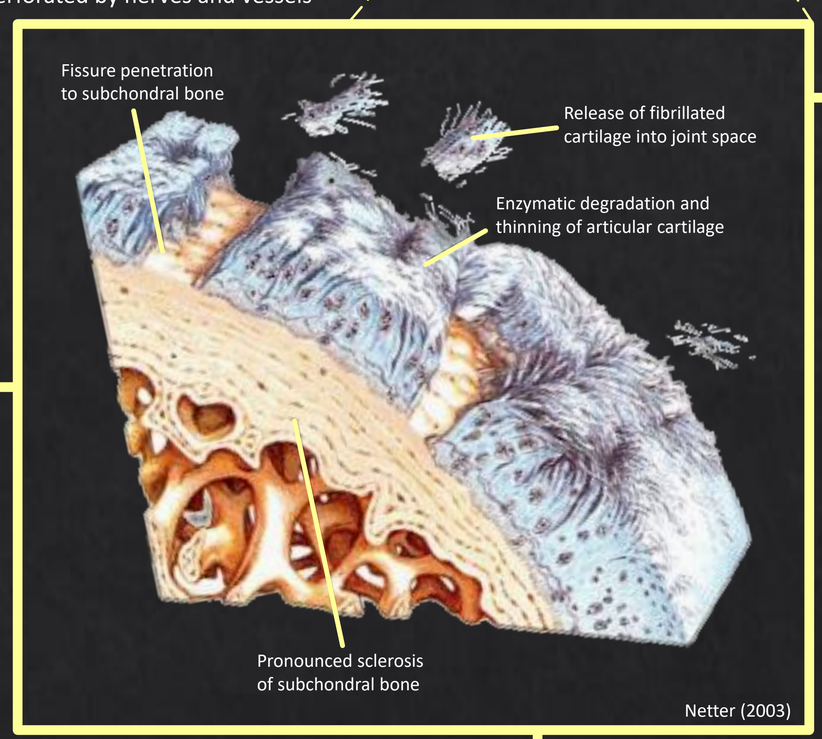


2.3 Limitations of current treatments:

Current treatments of OA are mainly symptomatic relief; there is no treatment that modifies the effects of OA and reverses the disease progression.

With the rapid growth and widespread adoption of 3D bioprinting in tissue engineering, we should consider the significant advantage of adopting 3D bioprinting in the treatment of OA.

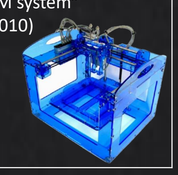




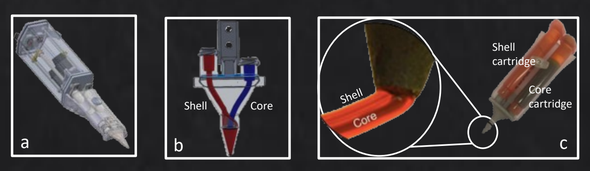
3. *In Situ* Bioprinting

Bioprinting is the technology of 'printing' or fabricating organs and living tissues. The 'ink' in bioprinting, also called the biomaterial, is actually living cells, with the addition of appropriate chemicals that induce growth and maturation of the cells. Computer-aided design (CAD) software is used to define the complex 3D structure of tissues and organs. Numerous studies have shown successful results of bioprinting functional cartilage, heart valves, bone and blood vessels among others.

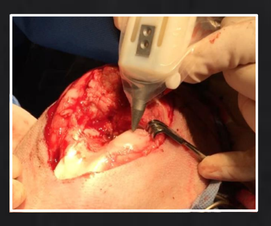
Bioprinting, in particular the direct printing of bioinks to repair living tissues at the defect site (*in situ* bioprinting), has a great potential as the future of joint repair. Two approaches of *in situ* bioprinting can be considered:

In Situ Bioprinting Approaches	Advantages	Example
Hand-held approach	• Smaller size; ease of control • Manually operated by surgeon – flexibility of navigating around native tissue • Lower cost	"Biopen" (O'Connell et al., 2016) 
Robotic arm approach, bench-based	• Can integrate advanced technology • Can achieve complex tissue architecture • Precise, automated	"Fab@Home AM system" (Cohen et al., 2010) 

3.2 Handheld approach:



a. Schematic representation of the 3D co-axial handheld printer
b. Schematic representation of the co-axial nozzle
c. Picture of the cartridges dedicated to Core and Shell loading in the printer, with relative magnification of the nozzle during co-axial deposition



By Bella et al. (2018) on a sheep chondral defect model using the handheld approach

a) Design:

The Biopen, designed by O'Connell et al. (2016) is a pen-like, hand-held device that allows extrusion of two types of biomaterial simultaneously, directly into the cartilage defect in a single-session surgery. The printing uses two inks from different chambers in a 'Core-Shell' manner: the 'core' bioink contains the stem cells with regenerative capacity, while the 'shell' bioink provides the scaffold with mechanical strength upon hardening.

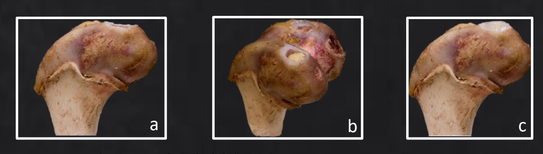
b) What was achieved in the study?

Full-thickness cartilage defects were created in the femoral condyles of sheep. The defects were treated in vivo using the Biopen, printing the cells and scaffold directly onto the defect. There were no postoperative complications. After 8 weeks, the 3D bioscaffold exhibited early cartilage regeneration with better overall macroscopic and microscopic characteristics, compared to preconstructed scaffolds.

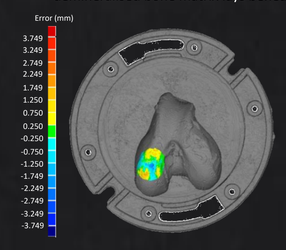
c) What potential does this study highlight?

The Biopen's technique of *in situ* bioprinting allows the free-form construction of tissue at the surgeon's discretion, in real-time in the operating theatre.

3.3 Robotic arm approach



a. Unrepaired osteochondral defect
b. Partially repaired osteochondral defect where bone portion has been printed
c. Fully repaired osteochondral defect where the hydrogel is visible and the demineralised bone matrix lays beneath



Top-view error plot of osteochondral defect. The laser scan of the printed surface was compared to the pre-damage CT scan surface geometry. Colours correspond to error magnitude.

Cohen et al. (2010)

a) Design:

The study used a lab-built extrusion-based bioprinter, with a robocasting-based additive manufacturing system (Fab@Home). A laser distance sensor was also accommodated to measure the geometric fidelity of printed constructs.

b) What was achieved in the study?

An osteochondral defect was introduced in a calf femur, involving both the cartilage layer and the underlying bone. Alginate hydrogel and demineralised bone matrix were used as bioinks in the *in situ* 3D printing. The repair print achieved a mean surface error of less than 0.1mm. Compared to the tolerances established for meniscal replacement procedures, the printed construct had 83.6% of surface points within clinically acceptable limits.

c) What potential does this study highlight?

This study demonstrates the possibility of achieving high precision assisted by geometric feedback and advanced path planning techniques, improving the outcomes of osteochondral repair with bioprinting.

4. Future Directions

Singh (2020)

Future of *in situ Bioprinting*

4.2 Limitations of Bioprinting

Limitation #1:

Optimising the selection of biomaterials remains a significant challenge. Whilst the polymers used in traditional 3D printing techniques have often proven too biologically active, causing unwanted cellular interactions and inappropriate stem cell differentiation, novel biopolymers and hydrogels, which more adequately mimic the microenvironment of the native tissue, often lack the structural integrity required for optimal bioprinting and can collapse if too soft. One promising solution has been combining substances to maximise the utility of each, namely the mechanical properties of a firm polymer, and the cytocompatibility of softer biopolymers and hydrogels.

Limitation #2:

Current anatomical understandings of cartilage substructure also represent a significant limitation in designing biomaterials to mimic cartilage. Whilst cartilage seemingly displays a simple, avascular and scarcely cellular structure, cartilage actually exhibits complex zonal architecture defining specific mechanical properties that are difficult to replicate artificially.

2019: Evolution of *in situ* Bioprinting

2007: Idea of *in situ* Bioprinting

4.3 Where to from here?

Future direction #1:

Enhanced bio-ink, containing smart nanocomposites, stimuli-responsive shape memory alloys (SMA) and shape memory polymers (SMP), is capable of reshaping post-printing in response to external stimuli, and may better model the dynamic mechanical properties of articular cartilage.

Future direction #2:

Integrative *in situ* bioprinting stations incorporate advanced scanning systems and adaptive closed-loop feedback control systems, which improve the ability to work on a dynamic printing surface, whilst also facilitating real-time correction of printing errors and non-compliant tissue. These machines are likely to be expensive, but represent a unique opportunity to leverage the automated precision of bench-based robotic arm printing in generating complex architecture, whilst also permitting dynamic surgical dexterity essential for greater customisation of the bioprinted construct.

5. References

Cohen, D., Lipton, J., Bonassar, L. and Lipson, H., 2010. Additive manufacturing for in situ repair of osteochondral defects. *Biofabrication*, 2(3), p.035004.

Di Bella, C., Duchi, S., O'Connell, C., Blanchard, R., Augustine, C., Yue, Z., Thompson, F., Richards, C., Beirne, S., Onofrillo, C., Bauquier, S., Ryan, S., Pivonka, P., Wallace, G. and Choong, P., 2017. In situ handheld three-dimensional bioprinting for cartilage regeneration. *Journal of Tissue Engineering and Regenerative Medicine*, 12(3), pp.611-621.

Runge, M., Greganti, M. and Netter, F., 2003. *Netter's Internal Medicine*. Teterboro, N.J.: Icon Learning Systems, p.856.

Singh, S., Choudhury, D., Yu, F., Mironov, V. and Naing, M., 2020. In situ bioprinting – Bioprinting from benchside to bedside?. *Acta Biomaterialia*, 101, pp.14-25.

Solomon, L., Warwick, D., Nayagam, S. and Apley, A., 2010. *Apley's System Of Orthopaedics And Fractures*. 9th ed. London: Hodder Education, pp.85-96.

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