Review article

Title: Hydrogel-based Drug Delivery for Tissue Engineering

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Abstract

Tissue engineering is a multidisciplinary field that aims to restore, maintain, and improve tissue performance by producing functional tissue constructs. The three major domains of tissue engineering are isolated cells or stem cells, biomaterial scaffolds and biomolecules. Hydrogels are commonly studied as scaffold materials for tissue regeneration due to its unique characteristics, i.e., excellent water absorbing and swelling capacity, tunable mechanical properties, porous structure, biocompatibility, biodegradability, flexibility similar to natural tissues and responsive to various stimuli (e.g., changes in pH, humidity, light and temperature). In the past decades, additive manufacturing (bioprinting) has emerged as a powerful technology in tissue and organ regeneration where it incorporates hydrogels as functional scaffolds. Various hydrogel-based delivery systems have also been developed in search of ideal scaffold for tissue engineering. In view of the potential of hydrogels in the field of tissue engineering, herein presents a review focusing on application of hydrogels for drug delivery in tissue engineering as well as additive manufacturing.

Keywords: bioprinting, hydrogels, smart hydrogels, tissue engineering, tissue repair and regeneration

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Introduction

Tissue engineering is a multidisciplinary field that aims to restore, maintain, and improve tissue performance by producing functional tissue constructs. In view of the limitations of current medical therapies for tissue injuries and shortage of transplantable organs, tissue engineering is regarded as the solution to these issues and has been explored extensively. Tissue engineering consists of three major components i.e., isolated cells or stem cells of the tissue of interest, biomaterial scaffolds, and biomolecules which are necessary for cellular development. [1]

Biomaterial scaffold is crucial for successful tissue engineering as it mimics the native extracellular matrices (ECM) to promote cell function, adhesion and tissue transplantation. Among the variety of biomaterial, hydrogels are widely used to fabricate scaffolds for tissue regeneration. Hydrogels are three-dimensional cross-linked polymeric networks with excellent water absorbing and swelling capacity while remain insoluble in the physiological environment. Other unique properties of hydrogels – excellent and modifiable mechanical properties, porous structure, biocompatibility, biodegradability, flexibility similar to natural tissues, responsive to various stimuli (e.g., changes in pH, humidity, light and temperature) – made it a promising candidate for tissue engineering. [2] To date, the application of hydrogel can be viewed in various tissue engineering and new advancements have been explored constantly to expand its applications in different tissue repair.

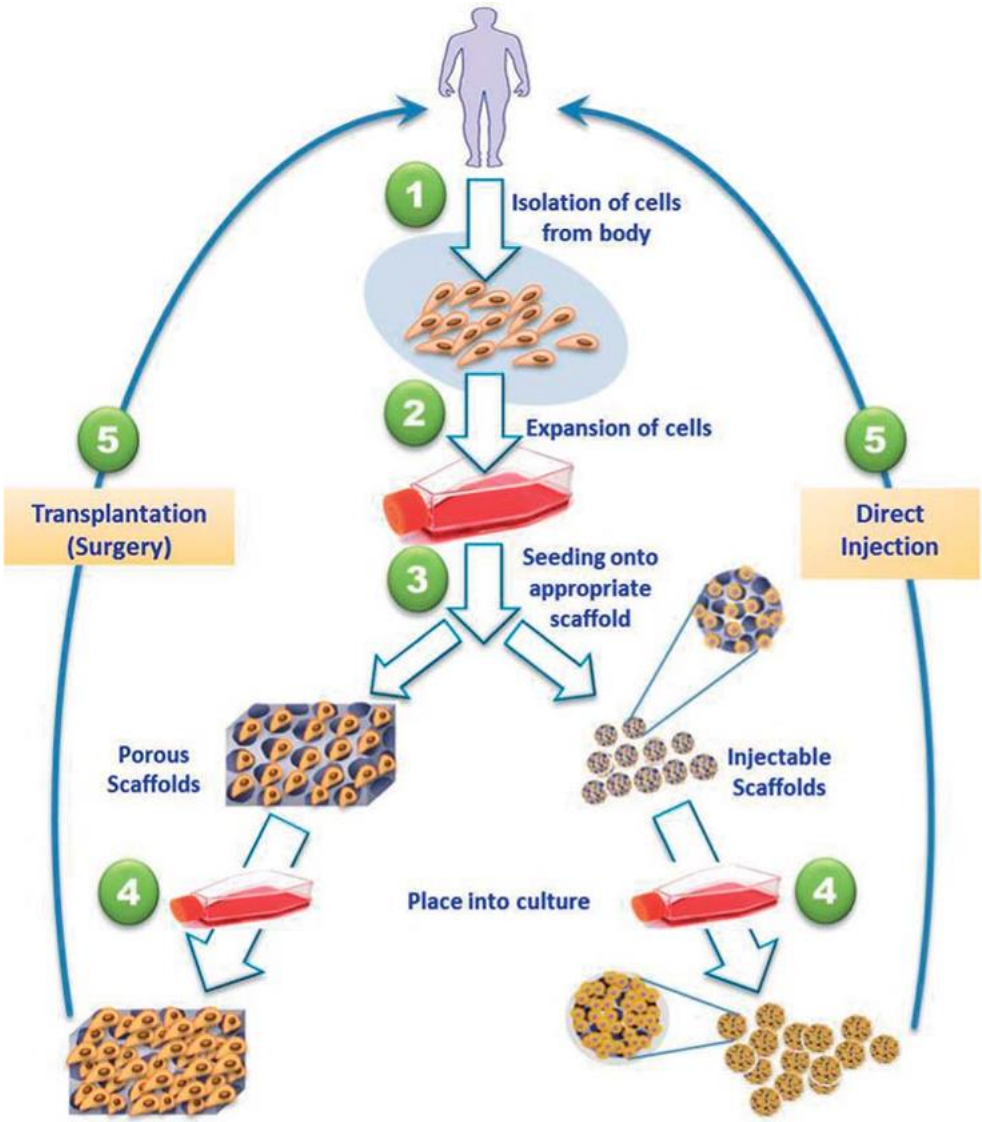

Figure 1. Commonly employed mechanism of tissue engineering. [1]

Characteristics of Hydrogel as Scaffold

Hydrogel is characterised with three-dimensional polymeric networks that is capable of absorbing substantial amounts of water and swells, while remaining insoluble in water and body fluid. The tremendous water absorption capacity of hydrogel is attributed to the hydrophilic side chain on the polymeric backbone while the dynamic cross-links between polymer chains maintain the integrity of the hydrogel network therefore resistant to dissolution. [2] Furthermore, hydrogel possess flexibility similar to the natural ECM to support the adhesion, engraftation, survival and proliferation of the neighbouring cells. [2]

Moreover, hydrogel is reasonably deformable and adaptive to the type of surface to which

they are applied, when combined with the bio-mucoadhesive nature of hydrogels can be utilised to confine them at the site of tissue injuries. [3]

Porosity is another critical property that permits the use of hydrogel in tissue engineering. Together with the high swelling structure, the interconnecting pores enable transfer of gases, nutrients, cell metabolites, and waste as well as assisting neovascularization to support growth of cells. [1] On top of that, the porous nature provides space for entrapment of biomolecules thus being utilised as carriers in sustained drug delivery system. [4]

An ideal scaffold should degrade in the later stage of tissue development to avoid hindering the complete tissue growth and confer a negligible inflammation extent. In view of these considerations, biodegradable and biocompatible hydrogels are therefore apt for tissue scaffold. [3] The mechanical properties of hydrogel can be tailored through modification of cross-linking of the polymer matrix, renders it to be modified for different tissue generation that normally demand different properties and functions of scaffolds. [1,2] Moreover, hydrogels can be made to respond to external stimuli by changing its mechanical structure. [3] The stimuli may be physical such as temperature, humidity and magnetic field, or chemical which include pH, ions and glucose. [2]

Hydrogels in Tissue Regeneration Technology

There are many tissue regeneration technologies being studied, among which, additive manufacturing utilises hydrogels to fabricate functional scaffolds.

Hydrogel for Three-Dimensional Bioprinting

Three-dimensional (3D) printing, also known as additive manufacturing, is a technology of building tissues or organs to reconstruct or replace damaged or defect tissues and organs. [5] The mechanism of 3D printing involved construction of tissue or organs layer-by-layer using a bottoms-up approach, guided by the computer model of the specific tissue or organ. 3D printings offer the unique advantages for tissue engineering as it can be customised to constructing tissues or organs with high structural complexity and is patient-specific. [5]

There are various 3D printing methods available currently, among which, bioprinting utilises hydrogel as bio-ink for the printing of tissue constructs. Hydrogel are highly preferred for 3D culture due to their printable, cross-linkable, biocompatible nature and high swelling capacity.

[5] In addition, the cells and other biological molecules can be mixed with bio-inks in the printing step thus creating a cell-encapsulated scaffold. Due to the combined advantages of 3D printing and hydrogel, 3D bioprinting has gained tremendous attention to generate 3D cell culture for tissue engineering. Recently, Tijore et al. [6] proved that the 3D bioprinted microchanneled gelatin hydrogel induced myocardial differentiation of stem cells and promoted the growth of cardiomyocytes, suggesting the possibility of in vivo generation of cell constructs for cardiac tissue engineering.

Hydrogel for Four-Dimensional Bioprinting

Apart from 3D printing, four-dimensional (4D) bioprinting has emerged as a more advanced approach for tissue engineering. 4D bioprinting employs a similar mechanism and offers the same advantages of 3D bioprinting. The difference lies in the incorporation of intelligent materials (e.g., smart hydrogels) which allows a 4D cell culture to respond to various stimuli and adapt to the changing environment consequently providing optimal environment for tissue regeneration. When applied with minimally invasive procedures (i.e., injection method), the cell cultures may access to sites normally difficult to reach, [5] When arrived at the target site and exposed with the specific stimuli, the scaffolds undergo stimuli-induced structural change (i.e., swelling, shape change and self-assembly) to allow adhesion and subsequent cellular development.

With the emergence of 4D printing, researchers have begun to explore its potential in tissue engineering. Recently, Miao et al. [7] has fabricated 4D smart scaffolds that showed excellent cell compatibility as well as active differentiation of human bone marrow mesenchymal stem cell on the printed scaffolds, indicating its potential for cardiac tissue engineering.

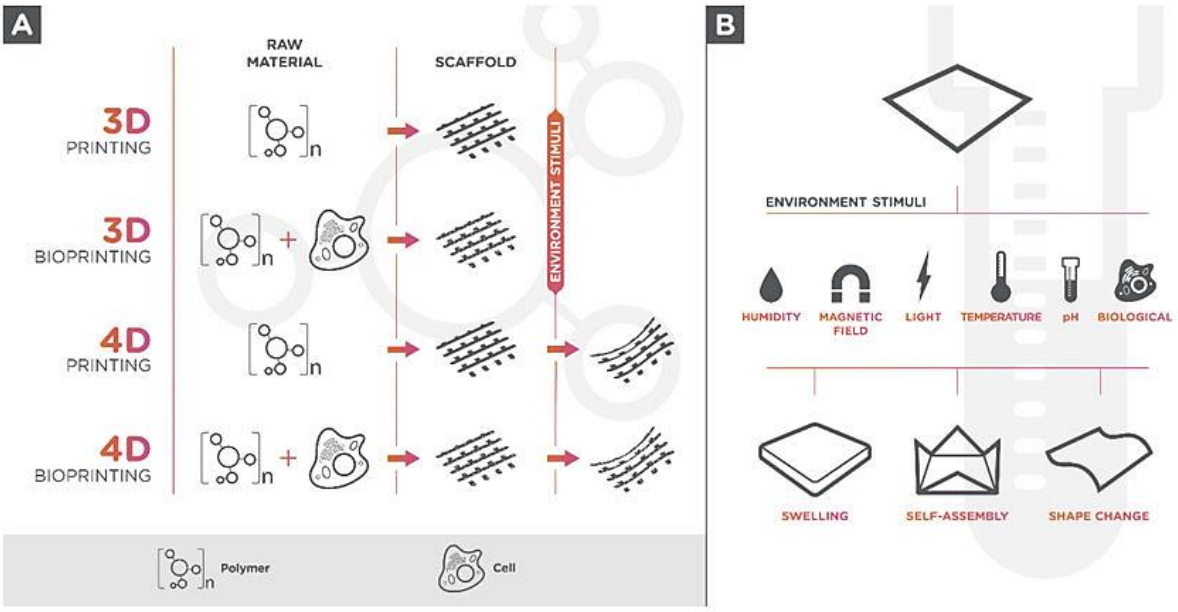

Figure 2. (A) 3D and 4D printing technologies to fabricate scaffolds; (B) structural changes in smart materials with exposure of external stimuli. [8]

Advancements in Hydrogels-based Drug Delivery for Tissue Engineering

To date, hydrogel has been experimented over and over and different delivery systems have been developed to accommodate for the need of ideal scaffold. Here highlights a few hydrogel-based drug delivery approaches which demonstrate promising function for tissue engineering.

Hydrogel-based Sustained Drug Released Systems

Tissue regenerations such as bone tissue repair and regeneration are complex and multiphasic processes that involve bioactive molecules of different levels. Hence, controlled sustained release of these bioactive components from the scaffolds has also been regarded as the key to create the optimal microenvironment and to achieve the desired tissue repair outcomes. In general, the drug release profile is highly dependent on parameters such as pore size, crosslinking density and degradation rate of the matrix. [9] As these parameters are manipulatable when designing hydrogel matrices, biomolecules can be formulated to have different release kinetics, i.e., in burst or controlled release.

Tremendous effort has been put into designing an ideal sustained-release system using hydrogel and plenty of research has demonstrated favourable results. These includes

controlled release of peptides (e.g., BMP) and growth factors (e.g., VGEF) [10], parathyroid hormone [11] and miRNA [12] from hydrogel matrix in bone tissue engineering.

As inflammation and immune response are inevitable and may jeopardise bone regeneration, incorporation of immunomodulatory drugs into the scaffold together with cells and other biomolecules is viewed as a solution to this concern. Zhang et al. [13] who fabricated a tetra-PEG hydrogel-based aspirin sustained release system has demonstrated reduced local inflammation, indicating that incorporation of aspirin abates the inflammation during bone regeneration which benefits the bone repair process. [13]

Nanocomposite Hydrogels

Nanocomposite hydrogels involve the incorporation of nanomaterials into the three-dimensional networks through physical or chemical crosslinking. The addition of nanoparticles is significant as it is capable of improving the physical and chemical properties of the scaffold, i.e., mechanical strength and toughness, protein and cell adhesiveness, elasticity, drug loading capacity, and degradation rate. [14] Various nanocomposite hydrogels have been developed to accommodate different functions for different tissue engineering (**Fig. 3**). For instance, Emami et al. [15] who fabricated a nanocomposite hydrogel based on gelatin/oxidised alginate has concluded incorporation of nanohydroxyapatite (nHA) provided self-healing property, high porosity, increased cytocompatibility, tunable gelling features in hydrogel for bone tissue regeneration.

Depending on the types of nanoparticles applied to the hydrogel network, additional and improved properties may be discovered from the nanocomposite hydrogels. An example depicting this is addition of zinc oxide nanoparticles (nZnO) into chitosan/gelatin hydrogel, the resultant hydrogel exhibits higher antimicrobial activity, lower cytotoxicity. [16] Such invention has showed great potential in skin tissue engineering as risk of infection is often concerned during skin repair and regeneration. As naproxen ionised at pH above 4.15, the positive charge of nZnO attracts the negatively charged drug molecules thus achieving a large load and continuous release of naproxen. [16]

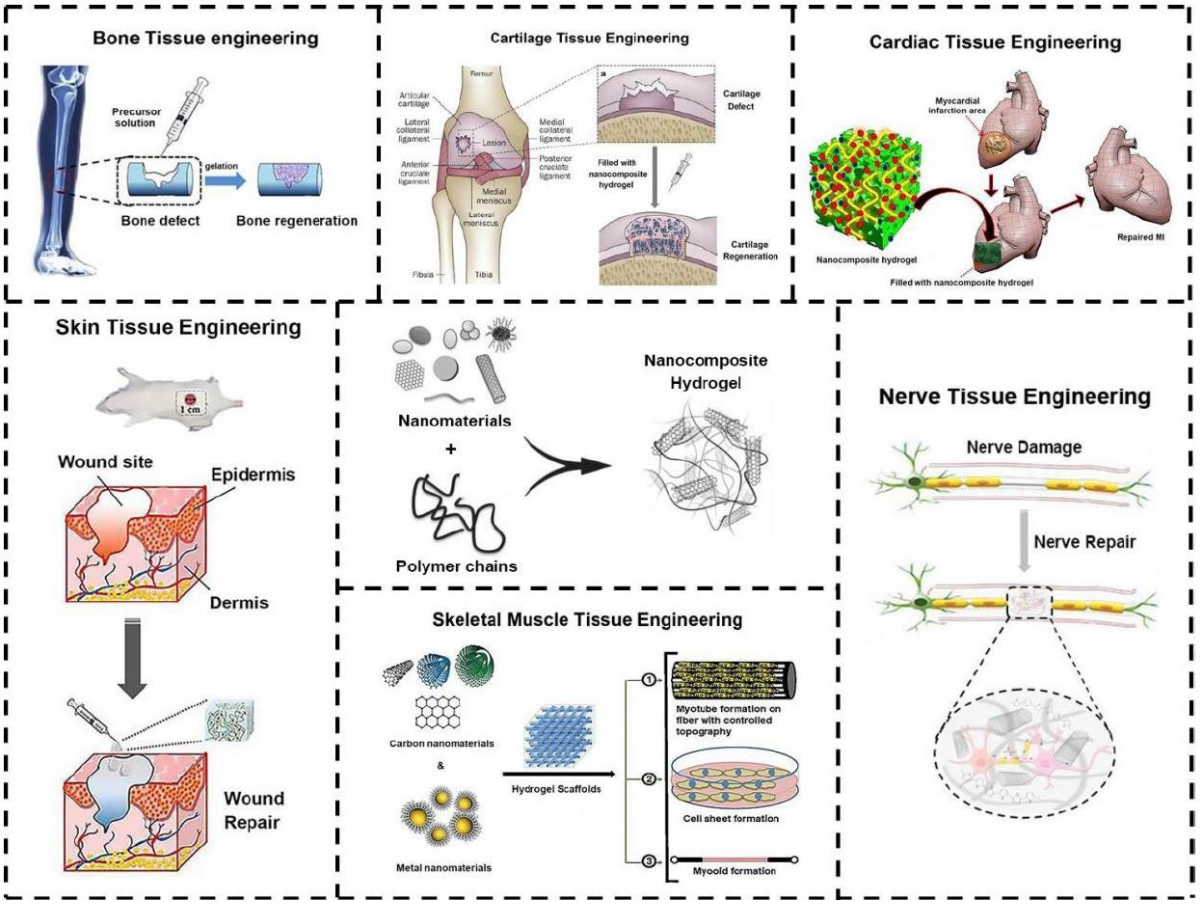

Figure 3. Various nanocomposite hydrogels were developed and studied for different tissue engineering. [14]

Stimuli-responsive hydrogels/ Smart hydrogels

Another notable characteristic of hydrogel is undoubtedly its ability to adapt by interchangeable sol-gel conditions which leads to the invention of smart hydrogel, also known as stimuli-responsive hydrogels (SRHs). Unlike conventional hydrogels, the design of smart hydrogel utilises surface-specific modification on polymer structure which allowing them to respond to various external stimuli. When exposed to the stimuli, the polymers react by changing the swelling behaviour, sol-gel transition, network structure, permeability, or mechanical strength. [3] Generally, smart hydrogels can be classified based on the stimuli and there are two broad categories of stimuli, i.e., physical and chemical stimuli. Examples of physical stimuli are temperature, magnetic fields, and humidity, while that of chemical stimuli includes pH and ions strength.

In the past decades, smart hydrogels have been extensively studied for the use in different

tissue regeneration. An example of smart hydrogels is the enzyme-mediated tissue adhesive hydrogels designed by Kim et al. [17] for meniscus repair of which tyrosinase was used to activate the tyrosine conjugated hyaluronic acid and gelatin hydrogels.

DNA-based Hydrogels

DNA-based hydrogel is a hybrid bionanomaterial developed from deoxyribosenucleic acid (DNA) and composed of cross-linked DNA chains in the 3D structure. In addition to the advantage confer by common hydrogel, DNA hydrogel preserves the biological features of DNA, i.e., sequence programmability, molecular recognition, excellent biocompatibility, and biodegradability. [18] As compared to traditional hydrogel, DNA-based hydrogels are superior in terms of self-assembly, programmable design and self-healing which is beneficial for cell migration. [18] In terms of encapsulation of cells, biomolecule and drugs, DNA-based hydrogels are capable of loading any other type of nucleic acid molecules (e.g., siRNA, miRNA) and DNA binding drugs as well as higher drug loading capacity. [18]

Moreover, DNA-hydrogel can be modified to be responsive to external stimuli, like smart hydrogels. The application of DNA-based hydrogels can be seen in various tissue engineering as well as tissue regeneration technology such as 3D bioprinting.

Recent Clinical Trial

In 2022, Niemeyer et al. [19] studied the treatment of large cartilage defects in the knee by hydrogel-based autologous chondrocyte implantation in a two-year, prospective, single arm, multicenter phase III trial involving 100 patients. The group had produced an albumin-hyaluronan-based hydrogel that is biocompatible and can be cross-linked in situ, to serve as a carrier in matrix-assisted autologous chondrocyte implantation (M-ACI). The administration of M-ACI was performed arthroscopically by NOVOCART® Injection plus. Due to the chondrocyte-loaded biomaterial's low viscosity and injectability, it encouraged homogenous cell distribution and excellent ingrowth of regenerated tissue into the defect bed. Moreover, the hydrogels were capable of preventing inflammatory and endothelial cell invasion, thus producing anti-inflammatory, anti-angiogenic, and thus also anti-osteogenic effects. [19]

The clinical outcome was assessed using the Knee Injury and Osteoarthritis Outcome Score (KOOS) on 3-, 6-, 12-, 18- and 24-months post operation (**Fig. 4**). Of which 93% of the patients score was at least 10 points higher, as early as 3 months after injection, indicating

significant improvement of the clinical symptoms. [19] Moreover, the repair tissue properties were assessed through Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) score and T2-mapping. [19] Both of which demonstrated progressive graft maturation and cartilage re-organization from 12 to 24 months suggesting minimal duration for the maturation of cartilage graft after ACI. [19]

Despite limitations in the research (e.g., lack of control group) and adverse reaction (e.g., arthralgia, joint swelling, and muscle dystrophy) of the formulation, the results still indicate the clinical significance of M-ACI with NOVOCART® Inject plus as a safe and efficacious treatment alternative for patients with large cartilage defects. [19]

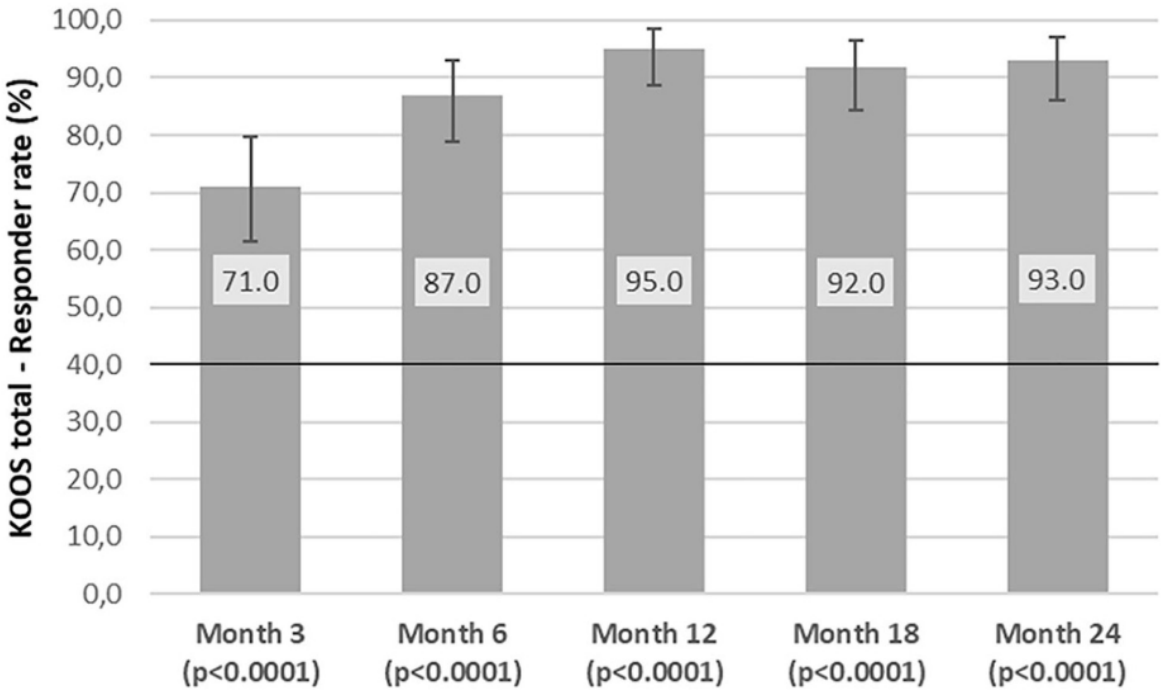

Figure 4. Knee Injury and Osteoarthritis Outcome Score (KOOS) responder rate on 3-, 6-, 12-, 18- and 24-months post operation.[19]

Conclusion

In view of the limitation of current medical and surgical interventions in treating severe tissue injuries, tissue engineering has been viewed as a promising alternative that attracts tremendous attention. To diversify the application of tissue engineering, hydrogel scaffolds that are characterised with tunable physicochemical properties have been experimented over different approaches, leading to new advancements with enhanced

functions as mentioned above. The combination of different types of hydrogels with latest additive manufacturing technologies is also demonstrated in 3D and 4D bioprinting. A clinical trial on M-ACI for cartilage defects also demonstrated clinically significance improvement. Although challenges remained in designing a perfect scaffold for tissue engineering, it is certain that, with more research, tissue engineering holds great potential to dominate the medical care in the future.

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