Biomaterials and Fabrication to Optimise Scaffold Properties for Musculoskeletal Tissue Engineering

Running title: Biomaterials and Scaffolds

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Abstract

Tissue engineering has emerged as a promising scientific field potentially yielding in vitro developed tissue to replace degenerative or injured tissues in vivo, thus avoiding the donor site morbidity associated with reconstructive surgery. Integral to the process is the role of scaffolds and the biomaterials used to form them. This review explores the concept of scaffold based tissue engineering and design considerations. The scaffold needs to have certain mechanical and architectural properties, it needs to be biocompatible and biodegradable, and allow combination with bioactive molecules. We also discuss scaffolding techniques, different biomaterial options and fabrication technologies, and future areas of development.

Key words: Tissue engineering; bioreactors; scaffolds; biomaterials; fabrication

1.Introduction

Population growth coupled with increasing life expectancy has increased the burden of orthopaedic pathology. As medical technology progresses it faces the demand of developing viable solutions to the processes of trauma and degeneration with resultant damage to biological tissues causing pain and impaired function. Current strategies of treatment encompass autografts (transplant of patients own tissue to sites of injury), allografts (transplant of tissue from one patient to another) and joint replacement procedures. Autografting is associated with donor site morbidity and restricted by the fact there is a limit to how much tissue can be taken from a donor site without compromising its function. Allografting has the inherent difficulties associated with immune system rejection and the mismatch between the number of patients and donors, and joint replacement is expensive and limited by implant survival issues.

Resultantly 'Tissue Engineering' has emerged as a 'multidisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ' (1-3). The essence of this field is the in vitro expansion of specific cells on porous matrices (scaffolds) to create three dimensional (3D) tissues that can be implanted into the site of tissue injury (4-6). Huge scientific interest has been attracted and research in this discipline has been myriad over the last twenty years with the seemingly unlimited possibilities it offers.

Integral to the process is the concept of the 'tissue engineering triad' composing of a) a scaffold which provides structure for tissue growth, b) a reservoir of cells to enable the development of tissue and c) growth stimulating signals (or 'bioreactors') to direct the subsequent interaction between cells and scaffold (7-9). Scaffolds are critical to the process as cells cultured in vitro form two dimensional sheets incompatible with 3D in vivo tissues, unless they are grown on 3D scaffolding.

A biomaterial is any substance, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces any tissue organ or function of the body. Scaffolds are therefore the biomaterial basis of tissue engineering and represent an area of intense research to develop a successful

application of tissue engineering which has so far been unrealised. This article will offer a review into the development and use of biomaterials as scaffolds in musculoskeletal tissue engineering.

2. Demands of a Scaffold

The structure of natural tissues serves as a guide to the design rationale within the field. A key concept is the role of extracellular matrix (ECM) which acts as an anchorage point for the majority of cells within the tissue – effectively forming a biological scaffold supporting cell proliferation and differentiation into mature tissue. The use of biomaterial scaffolds within tissue engineering is an attempt to mimic the ECM and facilitate cell mediated tissue regeneration (10, 11). Resultantly the characteristics of an effective scaffold parallel those of in vivo extra cellular matrix. These will vary depending on the tissue e.g. bone (12, 13), cartilage (14), meniscus (15), ligaments and tendons (16, 17). The limiting factor is the diversity of types of ECM within the body and their tissue specific composition (18-20) effectively curtailing the development of a single 'best fit' scaffold. Nevertheless it has been established that scaffold biomaterials should possess key characteristics as follows:

2.1 Mechanical and Architectural Properties

The scaffold must have sufficient mechanical strength to maintain the structure of the tissue into which it is implanted and the ability to resist the forces the tissue is routinely exposed to in vivo. This feature must be present from the time it is implanted until remodelling has taken place. Studies have shown that not only are mechanical properties imperative for implant survival the proliferating cells exhibit mechanosensitivity with scaffold stiffness influencing which cell lineages would preferentially differentiate on them in the case of stem cells (21) and which cell types adhere in the case of mature cells (22). The challenge in this regard is ensuring that the scaffold whilst being strong exhibits sufficient porosity in its structure to facilitate vascularisation, cellular penetration and efficient transport of oxygen, nutrients and waste products akin to the ECM. Equilibrium between these two design considerations is vital to the success of the scaffold.

2.2 Biocompatibility and Biodegradability

Key to the process is the need for the biomaterials used as scaffold material to be immunologically compatible to avoid an excessive inflammatory response and subsequent rejection and also should have high affinity for cells to allow them to interact and adhere to the scaffold. Scaffolds are not permanent implants and as such must be designed to be degradable allowing the body to replace the structure with its own tissue specific ECM. The rate of degradation should be similar to the rate at which new matrix is produced by the regenerating tissue to avoid mechanical collapse. Degradation occurs enzymatically or hydrolytically and can be influenced by scaffold design. It has been shown that scaffolds which are completely or partially degradable exhibit improved ECM distribution in comparison to non degradable scaffolds (23).

2.3 Manufacturing Considerations

The scaffold should be cost effective and there should be availability of batch production to make it viable in the clinical setting. Furthermore it should be possible to make it into varying shapes and sizes as demanded and consideration should be given to how it is packaged i.e. it should be amenable to sterilisation as per other surgical implants. The endeavour to develop a material and structure which most closely displays these characteristics is what steers current research with the ultimate aim of developing a clinically useful tissue engineering derived treatment. Scaffold design and development throughout the past decade can be considered both in terms of materials used and scaffolding technique, it is pertinent to consider both in reviewing the development of scaffolds.

3. Scaffolding Technique

Broadly speaking there have been four techniques employed throughout the period of research that are discussed in detail below. The advantages and disadvantages of each of these techniques are outlined in Table 1.

3.1 Pre-Constructed Porous Scaffolds

This represents the most established and widespread technique in which cells are ‘seeded’ in laboratory constructed porous scaffolds. The materials used are far reaching and can be broadly classified into three categories namely natural, synthetic and composite / semi-synthetic (24) which will be reviewed later. Examples of

naturally existing biomaterials are organic polymers including polysaccharides, inorganic ceramics such as calcium phosphates and more total constructs such as allograft derived ECM. Unfortunately despite displaying excellent biocompatibility they lack the required mechanical strength necessary for use in musculoskeletal tissue engineering. The composite biomaterials have subsequently been developed to address this short coming and are a combination of natural materials reinforced with synthetics (25) to improve mechanical strength. Synthetic biomaterials can be organic materials such as synthetic polymers like polylactic acid (PLA) and inorganic materials for example bioglass. The perceived advantage of these over natural materials is that their structure can tailored to give a wide range of mechanical and architectural possibilities, however this is somewhat negated by their lack of bicompatability inhibiting cell adhesion and proliferation on their surfaces. Again the composite materials which attempt to combine the positive properties of both have been developed to overcome this for example coating synthetic materials in collagen (26).

The technique has both strengths and weaknesses in comparison to others.
As the scaffolds are constructed in vitro the mechanical and architectural properties can be manipulated to develop scaffolds displaying analogous characteristics of the tissue specific ECM it is being implanted into. The technique also affords the use of a diverse range of biomaterials and as such affords flexibility in developing an appropriately designed scaffold, tailored to the varying demands of the tissue into which it is implanted. The major weakness is the difficulty of seeding the cells onto the scaffold in vitro. Limitations in the process can lead to unevenly distributed cells throughout its structure with the resultant construct displaying heterogeneous properties.

3.2 Cell Seeding of Allograft Derived ECM

This technique involves removing cellular antigens from allograft (or xenograft) tissues whilst preserving the ECM to develop immunologically tolerated scaffolds to which cells are then seeded in vitro. The ECM is decellularised by a variety of techniques including freeze thaw cycles and EDTA treatment (27). The decellularised ECM can then be used to replace an equivalent tissue to its base structure (28) or to

replace a tissue different from its native state (7, 29, 30). Advantages of this technique include its superb biocompatibility, the potential for preserved growth factors to stimulate cell proliferation on the scaffold and guide remodelling of the damaged tissue (31). The major disadvantage is that retained cellular components may stimulate an immune response and cell seeding difficulties and sequelae as for the previous technique.

3.3 Cell Sheets

This technique has been developed in Japan (32, 33) and involves the culture of cells on a temperature responsive polymer and inducing them to produce ECM and form into sheets. This can be repeated to produce multiple single layer sheets which can be bonded together to form thicker matrix. The structure of these matrices encourages neovascularisation much more readily than cell seeded scaffolds, however its value with regards musculoskeletal tissue engineering is limited as it would be very difficult to construct ECM rich tissues (due to the small volume of ECM produced by each sheet) which are typically found in bone and cartilage (12-14, 34). The technique is more applicable to tissues with high cell density such as corneal epithelium (35).

3.4 Encapsulation of Cells in Hydrogel Matrix

The principle of this technique is to ensnare living cells within a polymeric semi-permeable membrane which allows diffusion of nutrients and oxygen in and waste products out, whilst also preventing immune recognition of the encapsulated cells (36)**.** Typically hydrogels are used which are ‘cross linked polymeric networks which have the capacity to hold water within their porous structure’ (37). The driving force for its development has been transplant medicine with its applications including transplant of xenogenic pancreatic islet cells (38). The theoretical advantage of this technique is that the biomaterials used, from their base state as liquid monomers can be initiated to self construct solid 3D polymer meshes, with cells encapsulated (39).Enabling the complex to be delivered by injection as a liquid to the target tissue and initiated to set into the required shape. This potentially avoids open surgical procedures. So far its applicability for musculoskeletal tissue engineering has been limited by the poor mechanical properties of hydrogels.

4. Biomaterial Options

The biomaterial options which have been explored so far are broadly categorized into three groups namely, natural, synthetically derived or composite/ semi –synthetic (24). They have generally been modified for use in this domain from other established surgical uses including haemostatic agents, sutures and surgical site dressings (40). The review will focus on the established materials in these classes.

4.1 Synthetic Materials

The perceived advantage of this group is that they can be produced under controlled methods enabling bespoke design and producing predictable mechanical and physical properties. The most commonly used group is saturated aliphatic polyesters consisting of polylactic acid (PLA), polyglycolic acid (PGA), polylacticcoglycolide (PLGA)polycarprolactones (PCL). These polymers are degraded through hydrolytic de-esterification into monomers that are excreted via naturally occurring pathways and as such meet the demands of a biodegradable scaffold. There degradation rates and mechanical properties can be manipulated through the use of copolymers and adjusting molecular weight to satisfy the demand for specific structural characteristics for different applications. There is already emerging evidence of their successful application in tissue engineering as scaffolds for gene delivery (41) and more general applications (42). Polypropylene fumarate (PPF) is a linear polyester also with good biocompatibility and degradation parameters. It has the advantage over the other example polymers of potential use as an injectable material and has been explored as is initiated in situ to form a cross linked solid polymer. It has been explored for use in bone replacement tissue engineering (43) There are however limitations to these polymers with experimental data highlighting that they undergo bulk degradation which can lead to premature mechanical failure and the resultant degradation products can cause a strong inflammatory reaction further compromising their structure and ultimate success as a scaffold (44, 45). The combination of these polymers with ceramics such as hydroxyapatite is one strategy being explored to reduce the inflammatory response (46, 47).

Ceramics represent a large group particularly used in bone tissue engineering. They have been used in orthopaedic implants for many years and have been shown to have excellent bone bonding properties allied with excellent biocompatibility (48). The mechanism for this bone bonding is postulated to be through the formation of a carbonated hydroxyapatite (HCA) layer on the surface of the ceramic which acts as a biological interface with host tissue (49). This property lends itself to tissue engineering applications to prevent scaffold loosening following implantation. Furthermore ceramics have been shown to encourage vascularization, foster cell adhesion, growth and differentiation to osteoblasts and support enzymatic activity favouring success as tissue engineering scaffolds. (50-52) Examples of specific ceramics used as scaffolds include β-tricalcium phosphates, hydroxyapatite, Bioglass and calcium sulphate (12, 13, 53-56). The limitations of these materials are that they lack the desired mechanical properties needed to facilitate their use as load bearing scaffolds due to low compressive strength and fracture toughness.

4.2 Natural Materials

These materials represent nature-designed biological options which typically overcome the issue of bioactivity posed by synthetic polymers. Cells readily adhere to and proliferate on their surface and they are typically readily biodegradable. Collagen represents the most widely explored natural biomaterial. It is the most copious protein in mammalian tissue and a key component of extracellular matrix found in bone, tendon and cartilage – key tissues in musculoskeletal medicine (12-17, 57). It has been shown to readily allow cellular attachment and induce chemotaxis due to the topography of its surface - a key challenge when using synthetic materials and has seen it widely used on its own or in combination with other materials in tissue engineering applications (58-60). It effectiveness has been limited by concerns regarding transmission of infective diseases, inflammatory reactions, poor mechanical properties and uncontrolled biodegradability (61), the combination of collagen with synthetic materials to form composites has characterized the response to these issues (60, 61).

Polysaccharides are a further group of natural biomaterials, they have the ability to form hydrogels conferring the advantages described above. A key sub group is proteogylycans which make up one of the major macromolecules in articular cartilage

(14). Chitosan is an analog of this group and is derived from chitin which is found in arthropod skeletons. It has been shown to have bioactivity with chemoatrractive properties (62) and osteoconduction and is emerging as a biomaterial option (63). Further examples include starch (64), fibrin and decellularised extracellular matrix as described above (65). Despite their bioactive advantages the natural biomaterials pose challenges during fabrication resulting in heterogeneous structures and are difficult to mass produce consistently, furthermore they have proved to lack the required mechanical properties for load bearing musculoskeletal tissue engineering applications.

4.3 Composite / Semi Synthetic

This is the current focus of scaffold engineering. It involves the amalgamation of differing materials into a composite structure in an attempt to overcome the characteristic deficiencies of the individual constituents – i.e. combining materials having good mechanical properties and biodegradability with those displaying bioactivity. The main areas of research are bio-ceramics (combination of synthetic polymers with ceramics), synthetic and natural polymers combined and collagen amalgamated with synthetic polymers.

Examples include collagen/hydroxyapatite composites that has been demonstrated to induce bone formation and reabsorption similar to autologous bone transplant (59, 60, 65). PLA (polyactide-aliginate amalgam) (66) which has supported the chondro-induction of mesenchymal stem cells in vitro and collagen microsponges integrated into PGLA meshes (67).

5. Fabrication Technologies

This has formed a separate but equally important area of research to the choice of biomaterial. The overall goal is to create a mechanically strong and porous scaffold with a 3D structure to allow cell proliferation. The following options are the existing technologies, and their advantages and disadvantages are outlined in Table 1.

Different Fabrication Technologies	**Advantages**	**Disadvantages**
Solvent Casting and	Technically easy	Only creates thin sheets of

Particulate Leaching		material Solvents used can inhibit cell attachment and proliferation
Textile Methods	Technically easy Can be co-spun with other materials such as collagen	Difficulties in controlling pore size and rigidity of the scaffold
Phase Separation	Better control over topography and the characteristics of the material	Difficulty with cell survival necessitating composite scaffolds or combining it with other fabrication methods
Solid Freeform Fabrication / Rapid Prototyping	Internal micro-structure of the scaffolds can be specifically controlled	Cost Long time required to fabricate the scaffold

Table 1: Advantages and Disadvantages of different fabrication methods

5.1 Solvent Casting and Particulate Leaching

As a technique this involves combining the polymer biomaterial (dissolved in a solvent) with soluble particles and casting the composite into a 3D mould. The particulates are subsequently leached away via chemical reaction to create pores in the scaffold. The size of the pores can be controlled by the size of particles used (68). This technique is technically easy requiring non-specilaised equipment, however the limitations are that it can only be used to create thin sheets of material and that the solvents used can inhibit cell attachment and proliferation (69, 70).

5.2 Textile Methods

Woven and non-woven fibres can be bonded together using heat or adhesives as in fibre bonding (71) or electrospinning (72) can be used, which generates electrostatic forces to overcome surface tension of polymers to create a fibre jet. Commonly PGA and PLA are used and can be co-spun with other materials such as collagen to combine the advantages of both. Again these are relatively simple techniques but are limited by difficulties in controlling pore size and rigidity of the scaffold. PGA

scaffolds fabricated by these techniques have been used to engineer cartilage (14, 73) and tendon (16, 17, 74).

5.3 Phase Separation

This technique is based on changes in thermal energy to create separation of a homogenous polymer solution into a multi-phase system. With separation the solution separates into a polymer rich phase and a polymer lean phase. Subsequently solvent is extracted and this creates pores. As a result of the conditions created, the topography and the characteristics of the material can be controlled (75, 76). The process has been used to develop scaffolds in vitro for tissue engineering applications for bone and soft tissues (77, 78). Difficulties have been encountered with cell survival on scaffolds produced this way and this challenge has lead to the development of composite scaffolds using this technique and combining it with other fabrication methods in attempts to make them viable (79, 80).

5.4 Solid Freeform Fabrication / Rapid Prototyping

Computer data including computer aided design, CT and MRI data is used to create custom designs of 3D scaffolds using a variety of techniques such as 3D printing, selective laser sintering and stereolithography (81, 82). This technique is increasing in popularity as the internal micro-structure of the scaffolds can be specifically controlled – giving precise pore size, geometry and orientation allowing bespoke designs encouraging specific cell adhesion and propagation. The major limitations of the technique are the cost and the long time required to fabricate the scaffold compared to other techniques.

6. Combination with Bioactive Molecules

Both scaffold architecture and material can influence how scaffolds interact with cells an important consideration as explored above. An emerging further technique to improve bioactivity is to incorporate biologically active molecules into their structure (83, 84). The leading examples are through growth factor inclusion (85-88) and gene delivery (86) with the aim of influencing cell proliferation, differentiation, migration and gene expression to encourage tissue regeneration.

7. Current Status and Future Areas of Development

The review has summarised the current options available in terms of technique, biomaterial options and fabrication technologies. The plethora of existing options represents the myriad attempts to design a suitable bioactive scaffold that can be used clinically. The challenge is to balance the demands of required mechanical strength with architecture which has cell permissive internal structure and is sympathetic to the cellular response of the host tissue providing a suitable environment for tissue regeneration. So far the literature demonstrates very few examples of scaffolds that have been used clinically, spinal surgery has seen examples of scaffolds used in vivo with combination of recombinant human bone morphogenic protein-2 (rh BMP-2) with hydroxyapatite and tricalcium phosphate for spinal fusion with apparent success (87). Other examples exist on the market with limited data for example OSIGRAFT (Stryker) combining rhBMP-7 in a in a bovine collagen scaffold is indicated for delayed union of tibial fractures (88). Regarding systems designed for soft tissue structures treatment of rotator cuff tears has already seen scaffold based products implanted and tested. The results demonstrate no improvement in healing compared to standard treatment approaches highlighting the difficulties still faced in developing successful tissue engineering treatments (89, 90).

Research continues en-mass to develop the ideal material for scaffold applications with particular attention being paid to vascularisation strategies (91) bio-instructive and stimuli-responsive properties (92) and as delivery systems for growth factors and cytokines (92, 93) all factors which are increasingly being recognised as crucial to the survival, integration and ultimately success of scaffold based tissue engineered implants.

Conflicts of Interest

None

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None

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