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# Recent Advances and Current Developments in Tissue Scaffolding

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**Abstract:**

A bio-scaffold can be broadly termed as a structure used to substitute an organ either permanently or temporarily to restore functionality. The material that can be used varies with the application intended. Tissue Engineering is one such application demanding certain requirements to be met before it is applied. One of the applications in tissue engineering is the tissue scaffold, which provides either a permanent or temporary support to the damaged tissues/organ until the functionalities are restored. A biomaterial can exhibit specific interactions with cells that will lead to stereotyped responses. The use of a particular material and morphology depends on various factors such as Osteoinduction, Osteoconduction, angiogenesis, growth rates of cells and degradation rate of the material in case of temporary scaffolds etc. The current work reviews the state of art in tissue scaffolds and focuses on permanent scaffold materials and applications with a brief overview of temporary scaffold materials and their disadvantages.

## 1. Introduction and Background

The application of material in the field of medicine started as early as 17$^{th}$ century where wood was used to make artificial legs for restoring functionality of the legs. However, it was only during the last four decades the use of materials in medicine gained attention [119]. A significant development in the use of artificial materials in medicine was the Charnley's low friction arthoplasty [124]. The use of artificial materials often posed problems such as inflammatory response of surrounding tissues, need for secondary/revisional surgical procedure due to premature failure of the implants, stress shielding effects on the natural bone etc. The problems associated with the artificial organs directed research towards developing

materials/methods to restore functionality of organs with the assistance of minimally invasive surgical procedures. Tissue engineering is a multidisciplinary subject combining the principles of engineering, biology and chemistry to restore the functionality of damaged tissue/organ through repair or regeneration. The material used in tissue engineering or as a tissue scaffold can either be naturally derived or synthetic. Further classification can be made based on the nature of application such as permanent or temporary. A temporary structure is expected to provide the necessary support and assist in cell/tissue growth until the tissue/cell regains original shape and strength. These types of scaffolds are useful especially in case of young patients where the growth rates of tissues are higher and the use of an artificial organ to restore functionality is not desired. However, in case of older patients, temporary scaffolds fail to meet the requirements in most cases. These include poor mechanical strength, mismatch between the growth rate of tissues and the degradation rate of the said scaffold. Thus the older patients need to have a stronger scaffold, which can either be permanent or have a very low degradation rate. Due to the contrasting nature of requirements posed a judicial compromise between the properties requirement and the degradation rate has to be arrived at for the use of scaffolds in elderly patients [139]. Most of the works on scaffolding has been done on temporary scaffolds owing to the immediate advantages realized of the materials used and the ease of processing. Despite early success, tissue engineers have faced challenges in repairing/replacing tissues that serve predominantly biomechanical roles in the body. In fact, the properties of these tissues are critical to their proper function *in vivo*. In order for tissue engineers to effectively replace these load-bearing structures, they must address a number of significant questions on the interactions of engineered constructs with mechanical forces both *in vivo* and *in vitro* [1].

Once implanted in the body, engineered constructs of cells and matrices will be subjected to a complex biomechanical environment, consisting of time-varying changes in stresses, strains, fluid pressure, fluid flow and cellular deformation behavior [2]. It is now well accepted that these various physical factors have the capability to influence the biological activity of normal tissues and therefore may play an important role in the success or failure of engineered grafts [3,4]. In this regard, it would be important to better characterize the diverse array of physical signals that engineered cells may experience *in vivo* as well as their biological response to such potential stimuli. This information may provide an insight into the long-term capabilities of engineered constructs to maintain the proper cellular phenotype [5]. This paper attempts to present a brief over view on the tissue scaffolds both temporary and permanent, their processing and the present trends in scaffolding techniques.

## 2. Initial Applications of Tissue Scaffolding in Artificial organs

As explained above the tissue engineering or scaffold engineering techniques started with the use of artificial implants to restore functionality of damaged organ. The use of artificial implants started as early as the 17$^{th}$ century. Romans used legs made of wood to replace damaged legs/limbs to restore functionality. Thereafter the developments in the area of artificial implants were slow until 1962 when Charnley used low friction arthoplasty with Polytetra Flouroethylene (PTFE) to replace damaged joints. Subsequently, significant advances were made over the last four decades in the use of artificial implants. Various materials ranging metallic, ceramic and polymeric materials were used in artificial implants especially in the field of orthopedics. Stainless steel (surgical grade) was widely used in orthopedics and dentistry applications owing to its corrosion resistance. However later developments included the use of Co-Cr and Ti alloys owing to biocompatibility issues and bio inertness. Currently Ti alloys and Co-Cr alloys are the most widely used in joint prostheses and other biomedical applications such as dentistry and cardio-vascular applications. Despite the advantages of materials such as Ti and Co-Cr and their alloys in terms of biocompatibility and bio inertness, reports indicated failure due to wear and wear assisted corrosion. Ceramics was a good alternative to metallic implants but they too had their limitation in their usage. One of the biggest disadvantages of using metals and ceramics in implants was the difference in modulus compared to the natural bone. (The modulus of articular cartilage varies from 0.001-0.1 GPa while that of hard bone varies from 7-30 GPa). Typical modulus values of the most of the ceramic and metallic implants used lies above 70 GPa. This results in stress shielding effect on bones and tissues which otherwise is useful in keeping the tissue/bone functional [143]. Moreover rejection by the host tissue especially when toxic ions (in the alloy such as Vanadium in Ti alloy) are eluted causes discomfort in patients necessitating revisional operations to be performed. Polymers have modulus within the range of 0.001-0.1 GPa and have been used in medicine for long whose application ranges from artificial implants, i.e., acetabular cup, to drug delivery systems owing to the advantages of being chemically inert, biodegradability and possessing properties, which lies close to the cartilage properties. With the developments in the use of artificial implants there were growing concerns on the biocompatibility of the materials used for artificial implants and the immuno-rejection by the host cells. This led to the research on the repair and regeneration of damaged organs and tissues, which started in 1980 with use of autologuous (use of grafts from same species) skin grafts. Thereafter the field of tissue engineering has seen rapid developments from the use of synthetic materials to naturally derived materials that include use of autografts, allografts and xenografts for repair or regeneration of tissues.

## 3. Trends in tissue engineering Scaffolding

The use of engineered tissues dates back to as early as 1980's and has not matured yet. One of the reasons for its continued development is due to its multidisciplinary nature with varied requirements where it is necessary to combine the fields of engineering, medicine, chemistry and biology to arrive at the right kind of design or material. The primary aim of tissue engineering is to restore functionality of damaged organs/tissues from within the system instead of depending on artificial organs. Despite the early success in the use of Autografts, Allografts and Xenografts, which have good compatibility and recognition by the host tissue, there have been concerns about infection and second site morbidity of such tissues. In addition to the above donor scarcity necessitated further research in the field of Tissue Engineering to develop alternate methods. This called for the use of synthetic/naturally derived materials for scaffolding applications and seed the cells for growing natural tissue. The typical application of tissue engineering extends from human cells, tissues or organs to animal cells, tissues or organs.

The above-mentioned products are in different stages of development. In a little over a decade more than $3.5 billion is invested in worldwide research and development in tissue engineering. Many of the tissues and organs to be replaced/repaired serve important biomechanical functions and despite early successes, tissue engineers have faced challenges in repairing or replacing tissues [6]. Due to their complex nature and composition, most biological tissues can be classified from a material standpoint as inhomogeneous, viscoelastic, nonlinear and anisotropic materials. The fundamental basis for these behaviors is not fully understood, and may differ among different tissues. Also it remains to be determined, which aspects of these mechanical properties are essential for the normal, healthy functioning of different tissues and also for the replacements [7].

Assessment of the outcome of successful functional tissue engineering will require quantitative measures of graft properties, structure and composition. Given the biomechanical nature of many tissue-engineered products, there have been surprisingly few reports of the material or structural properties of engineered tissues. For example, several investigators have reported either mechanical property of grafts prior to implantation [8] or at sacrifice [9, 10]. An important direction in this field will be the development of new methodologies that will allow assessment of the material or structural properties of engineered tissues in a non-invasive or minimally invasive manner.

Hence the ultimate goal is to develop effective substitute or replacements for bone, cartilage, enamel, dentin, cementum and the periodontal ligament. Approaches for achieving this goal will consist of three strategies.

1. Using stem cells and their lineage to regenerate missing or damaged tissue *in vitro* or *in vivo*.
2. Developing new classes of biomaterials that may be either biologically derived or wholly synthetic.
3. Developing innovative physical/chemical stimuli to induce existing adult tissues to regenerate missing or damaged body parts.

It is important for an improved understanding of interfaces between cells, between cells and matrix, between different kinds of matrix and between cells, matrix and minerals. Interfaces between organic-inorganic compounds need to be more thoroughly investigated to determine the environment needed for physiological calcification and to achieve new approaches to designing hard tissues. Improved understanding of factors that affect regeneration and repair of tissues and the effect of nutrients, hormones, age and gender on such factors is needed. This kind of understanding will allow polymeric delivery systems to be developed and will provide new ways of influencing bone cells and other cell types.

Many of the present generation biomaterials are still based upon the early concept that implantable materials should be bioinert and therefore designed to evoke minimal tissue response, if none [11, 12]. However, a growing body of clinical data demonstrates that the long survivability of these materials is hampered by high rates of failure, which is primarily attributed to interfacial instability. Biomaterials developed should actively interact with tissues and thereby induce their repair/regeneration [13]. It is a far-gone conclusion that old cells must die and be replaced [14]. Biomaterials enable the repair and replacement of deteriorated bones and joints [15, 16]. The first generation of biomaterials selected was chemically inert in the body. These include, special medical grades of stainless steel, cobalt-chrome alloys, titanium alloys and polymer materials such as polyethylene and poly-methyl-methacrylate and are still the dominant materials in orthopedic surgery [17]. However such implants are reported to fail before the patient dies [18]. The reason for the failure is the progressive deterioration of the bone in contact with the biomaterial. Many factors contribute to the gradual breakdown of this interface; micromotion, wear debris, infection, stress-shielding etc. [19]. Creating new biomaterials is now the greatest challenges in material science.

## 4. Current Tissue Scaffold engineering techniques

As defined earlier in this paper scaffolds are constructs, which are used as a temporary support structure allowing the tissues/cells to adhere, proliferate and differentiate to form a healthy bone/tissue for restoring the functionality. The scaffolds can be classified in to two different categories based on their shelf life. The two categories are permanent and temporary implants. Permanent scaffolds those that retain their shape and strength through the process of regeneration/repair of the organ while the temporary scaffolds degrade over a period of time with the regeneration of the organ or tissue. Applications such as oral and maxillofacial surgery rely on the hard permanent scaffolds such as titanium-based scaffold for reconstruction or regeneration of the tissue/organ. However, research has been focused on development of scaffolds, which will be made of biodegradable and bioresorbable materials for most other tissue engineering applications. Temporary scaffolds used in tissue engineering can be broadly divided into two categories; synthetic or naturally derived, with a third category of semi-synthetic materials rapidly emerging. A particular challenge in addressing materials is that the processes are not yet completely understood well enough to allow a clear set of design parameters to be specified *a priori*. Adaptation of already used materials can have some advantages from the regulatory perspective, as the safety and toxicity profiles of the materials in humans are already defined, but other performance aspects such as cell material performance interactions and degradation properties however are not assured. This need for substantially higher performance characteristics is pushing research and development in the design of new materials that meet specific performance criteria in tissue engineering.

The field of biomaterials and scaffolds for tissue engineering is in an adolescent phase and maturing rapidly. One of the most important changes coming in the field is the strong need to integrate basic polymer science and engineering with molecular cell biology and stem cell biology in the design of new materials that carry out very sophisticated signaling needs.

The scaffold structures can be classified as adapted or designed. The structure of a scaffold plays an important role in guiding tissue development. Three very general types of scaffold are structural scaffolds with an imposed pore structure; gelatin type scaffolds formed *in situ* in the presence of cells or tissues, and natural tissue derived gels. For most tissues, the key requirement that can be defined at present is that interconnected porosity of larger dimensions than the cells is required or desired.

A variety of woven and non-woven fibrous fabrics, including plaster of Paris have been developed for tissue engineering purposes. Fibres are used because they present a large surface

area to volume ratio, which supports cell attachment, while creating paths for the rapid diffusion of nutrients for cell survival and growth. Tissue engineered bone; cartilage, heart valves, bladder and liver have been created with non-woven fibre scaffolds using rapid prototyping [20]. Research work is also currently being carried out on woven fibre technology for scaffold engineering using both natural (mostly collagen) and artificial polymer fibres [20]. The limitations of fabrics are lack of structural stability and possibly suboptimal microarchitecture, however this is now being catered for through hybridization in which sponges are combined with fibres to create scaffold [20]. In the case of artificial fibrous scaffold hot-drawing of PLLA fibres have been tried successfully to create structural stability by generating improved molecular orientation and crystallinity [21]. Furthermore, structural stability has been addressed by strengthening the joints of fibrous scaffold meshes with heated PLLA matrix [20,21].

The mechanical properties of degradable synthetic polymers are not always suitable for tissue engineering due to their relative inflexibility and tendency to crumble upon degradation. This has lead to development of additional polymers like polyhydroxybutyrate (PHB) and co-polymers of hydroxybutyrate with hydroxyvalerate [22]. They are included in this category due to their chemical simplicity and similarity to classical degradable polymers. The acidic degradation products of the classical polyesters Polylactic Acid (PLA), Polyglycolic Acid (PGA), and Polycaprolactene (PCL) and their copolymers have adverse tissue reactions, particularly in bony sites [24]. Hence there is need for improved biomaterials and scaffolds for tissue engineering. Some of the electron microscopy pictures of Collagen scaffolds and Confocal microscope image of PGA scaffolds is shown in Fig.1.

For regeneration of structural tissues, the scaffolds must possess several features that are difficult to achieve using current manufacturing methods [25]. Precise control of porosity and internal pore architecture is necessary to maximize nutrient diffusion, interstitial fluid [26] and blood flow [27], to control cell growth and function [28-31], to manipulate tissue differentiation [32-35], and to optimize scaffold mechanical function [36], and generated tissue mechanical properties [37]. The ability to manufacture with a range of materials is essential to control scaffold degradation and mechanical integrity, cells interaction with scaffold and cell function. To achieve this, a robust fabrication method that allows independent variation of scaffold structural parameters and materials are necessary.

Conventional sponge scaffold manufacturing methods are capable of producing structures with locally porous internal architectures from a diverse array of materials [38]. Local pores are

voids characteristically defined by small struts and plates or spherical and tubular cavities generally less than 300 μm in diameter. Local pores are interconnected within local regions of the scaffold microstructure. For example, porogen leaching yields cavities with defined shape [39-41] while an emulsion leads highly interconnected voids [42]. Solvent diffusion from emulsions can yield oriented pore structures [43,44]. Although these methods yield interconnected pores that may comprise a continuous conduit throughout a scaffold, the pore connectivity is not an intentional result of an *a priori* global design; rather, the connectivity is a random product of variable, local void interconnections that are affected by polymer processing parameters. Such random connections may not provide optimal scaffold permeability for tissue in growth nor optimal connectivity to maximize regenerated tissue mechanical properties [45]. Another problem associated with such highly interconnected pore architecture is the loss of strength and similar properties, which defeats the purpose of the scaffold in few cases.

Micro milling, textile and solid free form fabrication can be used for producing scaffolds with global pores [46, 47]. Global pores are channels with non-random interconnectivity and are generally greater than 100 μm in width. They are designed *a priori* to interconnect on a global scale across the scaffold. Previously, investigators have described scaffold pores based on size as either micro (width < 100 μm) or macro (width > 100 μm) [48, 49]. In contrast, global and local pores are discriminated based upon the extent of designed, not random pore interconnectivity. Zhang et al. [49] fabricated PLA scaffolds that contained stacked two-dimensional (2D) oriented global pores by leaching layered meshes made from drawn sugar fibers. While control over global pore size and 2D orientation was achieved, full control over global pore architecture is not possible without Solid Free Form (SFF) methods. Particularly, fabrication of interpenetrating pore systems that are mutually exclusive and pore channels that branch in three-dimensional (3D) space is impractical. Moreover, fabrication of scaffolds that mimic biological micro-structures is challenging. Such biomimetic scaffolds may prove invaluable to investigate structure-function relationships in living tissues and to replace native tissue function. [25, 51].

SFF scaffold manufacturing methods provide excellent control over scaffold external shape and internal pore interconnectivity and geometry, but offer limited micro-scale resolution. Currently, direct SFF fabrication is capable of producing global pores with a minimum feature size of 100 μm [52]. SFF fabrication, also known as rapid prototyping, consists of manufacturing a 3D part in a layered fashion based on a computer representation. The part is

often post processed (e.g. cleaning, curing, finishing) to yield the final product. During indirect SFF manufacturing, the part is used as a mould to cast the final product. These manufacturing techniques include: (1) fused deposition modeling (FDM of polymers) [53-55], (2) selective laser sintering (SLS) of polymers [56], ceramics [57], metals [58], and composites [59], (3) stereolithography (STL) of polymers and polymer/ceramic slurries [60-62], and (4) direct 3D printing (3DP) of polymers, ceramics [63], metals [64], and polymer ceramic powders [65]. Although the SFF technique has its advantages, the manufacturing processes limit the number of polymeric materials that can be used for direct SFF fabrication. For example, FDM and SLS require thermoplastic polymers, STL necessitates the use of radical initiated polymerization, and 3DP involve the use of solvents and binders. Additionally, direct SFF require multi faceted manufacturing control, including complex correction of scaffold designs for anisotropic shrinkage during fabrication. Incorporation of local pores under direct SFF is difficult because porogens or effusive solvents may interfere with the SFF method.

A group of researchers [140-146] attempted to use Titanium as a scaffold material in tissue engineering for applications demanding high mechanical strength and modulus. However, some of the typical problems indicated earlier such as stress shielding, premature failure due to poor wear properties, higher modulus compared to bone leaving to uneven stress distribution etc are associated with such scaffolds.

Composites of polymers and ceramics are noteworthy because the mixed material composition can confer favorable mechanical properties, including strength via the ceramic phase, toughness and plasticity via the polymer phase and graded mechanical stiffness. Biological advantages include enhanced control over cell differentiation and the potential to deliver multiple bio-factors, including growth factors gene therapy vectors and cells. Composite scaffolds may prove necessary for reconstruction of multi-tissue organs, tissue interfaces and structural tissue including bone, cartilage, tendon, ligament and muscle [66]. Materials in a composite may occupy the same volume as in a blend, occupy different volumes in one structure as in discrete composites, or may be combinations thereof. Composite fabrication research has focussed on developing polymer/ceramic blends, precipitating ceramic onto polymer templates [67-69] and coating polymers onto ceramics [70,71]. hydroxyapatite/poly(L)lactic (HA/PLA) blends have been made by melting and hot/cold pressing [72-75]. Solvent casting [76-79], in situ polymerization [80], and 3D printing. Additionally, sponges made from collagen and calcium phosphates or sintered bovine bone have been made via coating [81], gelation [82], or precipitation [83]. However, the mechanical property requirements for hard tissue repair are difficult to satisfy using blends because large

amounts of ceramic must be incorporated, making fabrication difficult. Kikuchi et al. found PLA must be reinforced with 75-80% HA powder to serve as a bone replacement [83]. Although HA reinforced polymer structures have shown improved strength and higher modulus proportional to increased HA content, the scaffold mechanical properties often become variable at HA concentrations above 70%. Therefore, composites with discrete regions of sintered HA are desirable for bone/soft tissue interfaces.

## 5. Naturally Derived Materials for Tissue Scaffolding

Lost or damaged genitourinary tissues have been reconstructed with native nonurologic tissues, examples are gastrointestinal segments [100, 101] skin [102], peritoneum [103] fascia [104] omentum, [105], dura or synthetic prostheses like silicone [106], polyvinyl and teflon.

Woven meshes of PGA (Dexon) have been used to reconstruct urethras in dogs. The polymer meshes were completely absorbed after 3 months. However the excised corpus spongiosum was not generated [107].

Collagen is the most abundant and ubiquitous structural protein in the body and may readily be purified from both animal and human tissues with enzyme treatment and salt/acid extraction [108]. Collagen has been known to elicit minimal inflammatory and antigenic responses [109]. This has been approved by the United States Food and Drug Administration (FDA) for many types of medical applications including wound dressing and artificial skin [110]. Collagen implants degrade through a sequential attack by iysosomal enzymes. The *in vivo* resorption rate can be regulated by control of the density of the implant and the extent of intermolecular cross-linking. The lower the density, the greater the interstitial space and generally the larger the pores for cell infiltration leading to a higher rate of implant degradation. Intermolecular cross-linking reduces the degradation rate by making the collagen molecules less susceptible to enzymatic attack [111]. Inter molecular cross-linking can be accomplished by various physical or chemical techniques [112]. Collagen contains cell adhesion domain sequences that elicit specific cellular interactions. This may assist in retention of the phenotype and activity of many types of cells, including fibroblasts [113] and chondrocytes [114]. Collagen exhibits high tensile strength and flexibility and these mechanical properties can be further enhanced by intermolecular cross-linking. This material can be processed into a wide variety of structures like fibers, films etc. Methods for stabilizing collagen-based materials with catechol containing monomers were developed in order to produce fibers with mechanical properties in tension comparable to those of normal tendon.

Alginate, a polysaccharide isolated from seaweed has been used as an injectable cell-delivery vehicle [115] Alginate is biocompatible and has been approved by the FDA for human use as a wound-dressing material. Alginate is a family of copolymers of D-mannuronate and l-glucuronate. The physical and mechanical properties of alginate gel are strongly correlated with the proportion and length of the ploygluc uronate block in the alginate chains [115]**.** However, alginate does not posses a biologic recognition domain. In addition the range of mechanical properties available from alginate hydrogels is quite limited and changes in a concontrollable manner, presumably due to loss of ionic cross-linking. Recently, efforts were made to synthesize biodegradable alginate hydrogels with mechanical properties controllable in a wide range by intermolecular covalent cross-linking and with cell adhesion peptides coupled to their backbones [116].

Acecellular tissue matrices are collagen rich matrices prepared by mechanical and chemical manipulation of a segment of bladder tissue [117]. The matrices slowly degrade upon implantation and are replaced and remodeled by Extra Cellular Matrices (ECM) proteins synthesized and secreted by transplanted or in growing cells. Acellular tissue matrices have been proven to support cell in growth and regeneration of genitourinary tissues, including urethra and bladder tissues, with no evidence of immunogenic rejection [118]. Since the structure of the proteins in acellular matrices are well conserved and normally arranged, the mechanical properties of the acellular matrices are not significantly different from those of native bladder submucosa [119].

The effect of temporary encapsulation of rat marrow stromal osteoblasts in cross-linked gelatin micro particles on cell viability and proliferation was investigated by Gregory et al. [120] for micro particles placed on a cross-linking poly propylene fumarate (PPF) composite over a 7 day time period. Encapsulated cells were seeded on cross-linking PPF composites at times up to 10 min following initiation of the cross-linking reaction, and also on fully cross-linked PPF composites and tissue culture polystyrene controls, with a cell seeding density of $5.3 \times 10^4$ cells/cm$^2$. The cross-linked PPF composite exhibited an average gel point of 10.3 min and an average maximum cross-linking temperature of $47.5^0$C. Cell viability and proliferation were assessed by DNA and 3H-thymidine assays and the results were compared with those for non encapsulated cells. The results showed that the addition time of cells to a cross-linking PPF composite had a large effect on cell viability and proliferation for both encapsulated and non encapsulated cells with more surviving time points. The study indicated that the presence of gelatin micro particles does not affect the cross-linking of a PPF composite. They further suggest that the temporary encapsulation of cells in cross-linked gelatin micro particles may

preserve the viability of cells contained in an actively cross-linking PPF composite used as an injectable polymeric scaffold serving also as a carrier for osteogenic cell populations.

Silk from the silkworm, Bombyx Mori, has been used as biomedical suture material for centuries. The unique mechanical properties of these fibers provided important clinical repair options for many applications. During the past 20 years, some biocompatibility problems have been reported for silkworm silk; however, contamination from residual sericin (glue-like proteins) was the likely cause. More recent studies with well-defined silkworm silk fibers and films suggest that the core silk fibroin fibers exhibit comparable biocompatibility *in vitro* and *in vivo* with other commonly used biomaterials such as polylactic acid and collagen. Furthermore, the unique mechanical properties of the silk fibers, the diversity of side chain chemistries for `decoration' with growth and adhesion factors, and the ability to genetically tailor the protein provide additional rationale for the exploration of this family of fibrous proteins for biomaterial applications. For example, in designing scaffolds for tissue engineering these properties are particularly relevant and recent results with bone and ligament formation in vitro support the potential role for this biomaterial in future applications. To date, studies with silks to address biomaterial and matrix scaffold needs have focused on silkworm silk. With the diversity of silk-like fibrous proteins from spiders and insects, a range of native or bioengineered variants can be expected for application to a diverse set of clinical needs [121]. Some of the examples of naturl and synthetic scaffolds are shown in Fig.2

Hydrogels, cross-linked hydrophilic polymers, represent an important class of biomaterials in biotechnology and medicine because many hydrogels exhibit excellent biocompatability, causing minimal inflammatory responses, thrombosis and tissue damage [124]. Hydrogels can also swell large quantities of water without the dissolution of the polymer due to their hydrophilic but cross-linked structure, thus giving them physical characteristics similar to soft tissues. In addition, hydrogels have high permeability of oxygen, nutrients, and other water-soluble metabolites. Over the past three decades, a number of hydrogels differing in structure, composition and properties have been developed. These materials are used for biosensors, linings and scaffold devices [125]. Photopolymerized hydrogels have been investigated for a number of biomedical applications like drug delivery [127], coatings for biosensors [128] and for cell transplantation[129-131]. Photpolymerizable hydrogels have been utilized as scaffolds for vascular cell growth. Endothelial cells in biodegradable poly(propylene fumarate-c-ethyleneglycol) scaffolds were implanted subcutaneously Mann et. al. in rats [132]. These materials were PEG-diacrylate derivatives with grafted RGD-peptides. The cells were homogeneously distributed within the scaffolds and remained viable with subsequent

proliferation and matrix protein production. In proteolytically degradable scaffolds, cells were able to spread and migrate, while in non-degradable hydrogels, cells were round and formed clusters. Additionally, cell proliferation and extracellular matrix production reached higher levels in proteolytically degradable hydrogels than in similar non-degradable PEG-diacrylate scaffolds [134].

The study conducted by Masatoshi et al. [134] investigated whether three biodegradable materials, poly-N-acetyl-D-glucosamine (chitin), poly-ε-caprolactone (p-CL), polylactic acid (PLA) and chitin/p-CL composite could be used as scaffold implants in the reconstruction of extra-articular ligaments or tendons. With chitin tendons, there was good tissue in growth among the chitin fibers. However, because of the rapid loss of strength *in vivo* and the inability to maintain sufficient strength, chitin cannot be used alone as an artificial tendon. p-CL had stronger load at failure than chitin, but it scarcely induced the formation of new fibrous tissues, so p-CL alone is also unsuitable as an artificial tendon. Conversely, both PLA tendons and the composite chitin/p-CL tendons had good initial strength and showed increased in growth of fibrous tissue, suggesting that these materials are promising as artificial tendons.

## 6. Bioactive Materials Used in Tissue Engineering.

Bioactive glasses composed of 45 to 52% $SiO_2$, 20 to 24% CaO, 20 to 24% $Na_2O$ and 6% $P_2O_5$ have been successfully used in clinical practice [85-87]. The characteristics of this Class A bioactive materials are good bonding with both bone and soft connective tissues, both osteoproductive (colonization and proliferation of bone distant from and implantation site) [88] and osteoconductive (growth of bone along the surface of the material) [88, 89], and finally the material resorbs as new bone replaces it. A particulate form of the 45S5 composition, called Bioglass has been used to regenerate bone in periodontal defects [90].

Synthetic hydroxyapatite (HA) is an example of a Class B bioactive material. Its characteristics are bonding with bones but not to soft tissues, osteoproductive and it resorbs very slowly if at all in contact with newly forming bones. Osteoconductivity is an essential property of porous hydroxyapatite, which leads to replication of a trabecular bone structure [91]. This characteristic has led to the clinical applications to fill large bone defects. This is sold commercially as “Interpore”.

Until recent experiments by Hench and others [13, 89] little was known about the molecular mechanisms responsible for enhanced bone proliferation and differentiation; i. e. enhanced osteogenesis, stimulated by the presence of Class A bioactive glasses [93]. Previous studies in cell culture established that oestoblasts from various sources grown on 45S5 Bioglass

increased in number and produced more phenotype markers, such as alkaline phosphate, than did equivalent osteoblasts grow on metal substrates such as stainless steel or Ti, plastics or even Class B bioactive materials such as synthetic hydroxyapatite [16, 94-96]. Other cell culture studies of primary human osteoblasts grown in the presence of certain silicate materials, Zeolite A, or either extracts showed an enhanced production of a class of growth factors compared with control materials [97]. Other osteoblast culture studies showed evidence of enhanced extracellular matrix production and even nodule formation in the presence of bioactive glasses [98]. However none of the studies has reported that it is the shift in cell cycle that is responsible for the enhanced osteoblasts are exposed to Class A bioactive materials. Studies by Hench et al., [13, 90] have shown that the bioactive shift of osteoblast cell cycle in under genetic control. It seems that Class A bioactive glasses enhance osteogenesis through a direct control over genes that regulate cell cycle induction and progression. This conclusion provides hope that it will be possible to deliver a critical concentration of the bioactive genetic stimuli to reactivate the osteoprogenitor cells of aging people and prevent or perhaps even reverse the deterioration of bone. If this can be achieved it will be a remarkable innovation in biomaterial research.

## 7. Scaffolds for Hard Tissue and Bone Reconstructions

Though significant advances have been made in scaffold fabrication for soft tissue replacement using degradable synthetic polymers or natural polymers, hard tissue replacements have posed a potential challenge to biomedical engineers and surgeons. Current practice to repair/ regenerate hard tissues is to use autografts. However, due to limitations on the availability, second site morbidity of tissues and patient associated problems alternative treatment methods are being researched on Strength analysis of osteotomy fixation performed by Marcelo et al [137] indicated that the resorbable systems (polyurethane) possess lower elastic stiffness and modulus compared to permanent Titanium systems. The resorbable systems showed isolated failure for in-vitro testing and despite their ability to withstand the mastication forces in-vitro, the in-vivo behavior is yet to be successfully tested. Though the resorbable systems can be used for scaffolding in soft tissue regeneration, hard tissues necessarily require bone grafting using either autografts or xenografts. An alternative to this is to use hard titanium based implant with a bioactive surface. A typical problem associated with this type of implant is the stress shielding and adverse tissue reactions with the alloying additions used due to micro movements.

Research has also been focused on use of porous Titanium scaffolds for tissue engineering applications [140-146] and induction of bioactivity by using secondary processing such as coatings. This type of porous Titanium scaffolds have received considerable interest in the recent past owing to their advantages over biodegradable matrices such as PLA/PGA and other associated polymeric materials. Titanium and its alloys have been widely used as scaffolds for maxillofacial and craniofacial reconstructions over the past two decades. Besides this Titanium mesh in the form of fenestrated sheets have been used in the acetabular replacement surgery [138]. Titanium meshes were first used by Jurgen harms and Lurtz Biederman in 1986 for spinaly surgery forming in to different shapes to support the bony structure [139]. This design was further modified to provide the strength to the construct. The Titanium mesh cage contoured in to cylindrical shape has been used successfully for Anterior Lumbar Interbody fusion for more than 15 years in surgery. Titanium mesh cages were used with autografts for bone grafting in spinal fusion. This is restricted by factors such as complications and second site morbidity. One method to overcome this problem is the use of hydroxyapatite to provide the necessary bioactivity to the Titanium mesh cage with porous network to facilitate osteoconduction. This method has been found to be successful and a viable alternative to autograft or femoral ring allografting methods [140].

## 8. Surface Architecture and Terrain for Cell Culture for Scaffolding

Surface terrain or topography is one of the important factors governing cell adhesion and proliferation, and there has been many studies carried out in recent research to investigate the suitability of materials such as spider webs and cover slips, fish scales, plasma clots, and glass fibres [135]. Silk fibres have also been used extensively in surgical implant such as suture and artificial blood vessels [136]. Few researchers [135,150-153] studied the applicability of cellulose roughness as a possible texture for tissue scaffold and successfully cultured hepatocytes cells. Cell adhesion to materials is mediated by cell-surface receptors, interacting with cell adhesion proteins bound to the material surface. In aiming to promote receptor mediated cell adhesion the polymer surface should mimic the ECM. ECM proteins, which are known to have the capacity to regulate such cell behaviors as adhesion, spreading, growth, and migration, have been studied extensively to enhance cell-material interactions for both *in vivo* and *in vitro* applications [150, 151]. However, the effects observed for a given protein have been found to vary substantially depending on the nature of the underlying substrate and the method of immobilization [152, 153]. In biomaterial research there is a strong interest in new materials, which combine the required mechanical properties with improved biocompatibility. Instead of developing new biomaterials, surface coating or surface modification presents a way

to preserve the mechanical properties of established materials and to improve the surface biocompatibility [154]. A major objective in the design of fictionalized polymer surfaces for biomaterial applications is the attachment of proteins or peptides that promote integrin-mediated cell attachment. Most of the conventional materials do not meet the demands required for both their surface and bulk properties when used as biomaterials. An effective approach for developing a clinically applicable biomaterial is to modify the surface of a material, that already exhibits excellent biofunctionality and bulk properties.

Many materials used in surface engineering lack in reactive groups for the covalent attachment. For these materials the major challenge of surface engineering is the introduction of reactive groups to the surface. Methods of surface engineering and surface covalent attachment have been discussed in a number of excellent reviews [155]. These studies are of significance for future work on the surface engineering of scaffold and we note that they are establishing the initial stages of the application of biomimetics in the design and manufacture of tissue engineering scaffolds.

## 9. Conclusions and Future Directions for Tissue Engineering Scaffolds

Research in tissue engineering has revealed the design rules for joining cells with materials and for understanding the mechanism by which cellular functions can be influenced or interrogated by materials. Development of cell based microsystems outside tissue engineering will in the long term provide technologies that will be applied to tissue engineering. The technology developed to integrate the functions of cells with electrical or mechanical processes in materials will have important benefits to the growth of tissue for transplantation and for prosthetic interfaces between indwelling devices and natural tissue. Cell based engineering addresses the development of hybrid devices that combine cellular and tissue components with conventional materials and processes found in microfabrication. Research and development activities span a broad range of topics including technical development of methods and fabrication routes to join cells with materials, exploratory and discovery research to identify strategies for matching cellular processes with materials processes, and engineering of complete systems that exploit the unique realization that combining man made devices and biological systems.

Despite the advancements in the field of tissue engineering and scaffolds for tissue engineering, there are few challenges, which needs to be addressed. Various types of materials have been used for application in artificial implants or scaffolds can be broadly classified in to six categories namely, biotoxic, bioinert, biodegradable, bioresorbable and bioactive. Of these

most artificial implant materials either fall under the biotoxic (cadmium, vanadium, mercury used as dental amalgams) or the bioinert (tantalum, titanium etc.) categories while biodegradable, bioactive and bioresorbable materials are used in tissue engineering either singly or in combination or with bioinert materials depending on the application. The type of challenge varies with the material used for scaffolding application. Issues that needs to be addressed in temporary scaffolds using biodegradable polymers includes mechanical strength, toxicity of degradation products to the host tissue (If acidic polymer is used it may release acidic degradation products which is harmful to surrounding tissues), interconnected pore architecture for supply of nutrients upon cell seeding and smart control of degradation rate of the scaffold. Scaffolds fabricated of bioresorbable and naturally derived materials suffer from lack of mechanical strength and processability. Permanent scaffolds fabricated of metallic/ ceramic materials can overcome the limitations with respect to strength and toxicity. Bioactive titanium meshes have been successfully used in spine fusion surgery for the past two decades. Most Ti mesh cage uses autograft bones or femoral ring allograft for bone grafting. As explained earlier in this paper both the techniques have disadvantages such as complications during surgery, second site morbidity, infections in case of allografts and immunological rejections. Synthetic hydroxyapatite and coralline hydroxyapatite has been found to be a good alternative to autografts and allografts as they provide the necessary osteocondution. However, the effect of pore morphology in the mesh and the mesh architecture has not been given a serious attention in the past. The stress shielding effect should be minimized if not eliminated by designing the mesh architecture, which would be conducive to osteoconduction in the presence of bioactive substances.

In the near term, a central challenge in these programs is the development of a common framework for designing and building structures having both materials and biological components. This framework must address the development of strategies to integrate the functions of engineered systems which are based on firm physics and engineering, use inorganic and metallic materials and are constructed with photolithography and micro-fabrication tools, with the functions of biological systems which use soft materials in aqueous environments, rely on self assembly for their construction and where the design rules are in many cases incompletely understood. Clearly, the field of tissue engineering needs to establish functional criteria that will help those who seek to design and manufacture these repairs and replacements. Scale-up, packaging, storage and handling properties are also critical. The implants must be capable of retaining their mechanical, structural, and biological integrity during surgical implantation. Understanding those conditions that preserve the character of the

implants may be essential for the success of the tissue-engineered products. It is important to consider the principles of functional tissue engineering in the light of the role of novel growth factors and new biomaterials.

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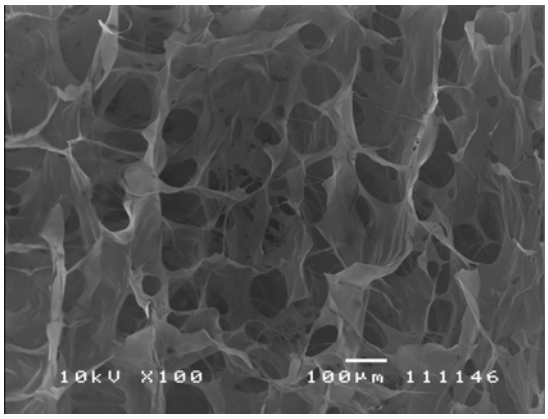
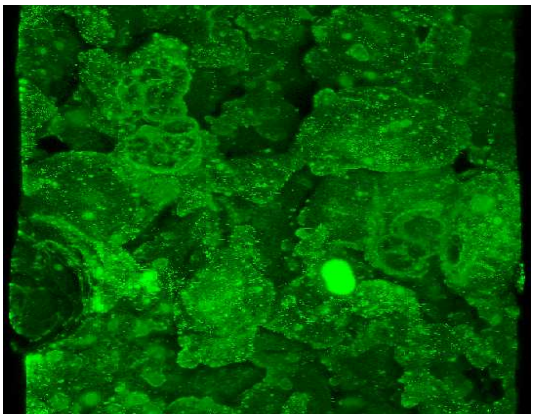


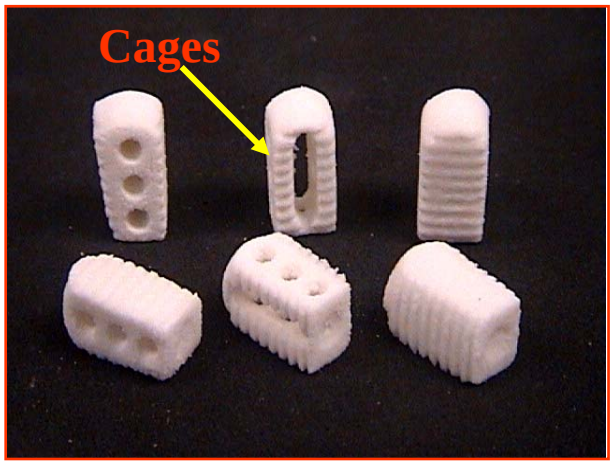

(a) SEM picture of bovine Collagen scaffold (courtesy Dr. Philip Cheang,/Dr. Ma NTU)

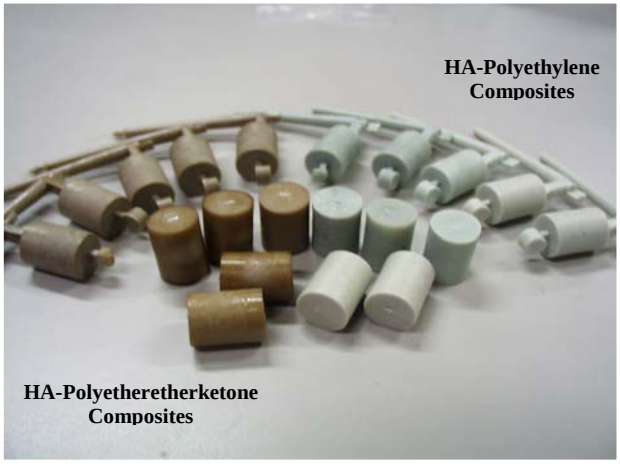


(b) Confocal microscope image of PGA scaffold

Fig. 1 Microscopic Images of Scaffolds

(a) Bioglass implants/scaffolds : Courtesy Dr. Philip Cheang, NTU




(b) Biocomposites using synthetic polymer and HA for scaffold applications: Coutesy Dr. Philip Cheang, NTU

Fig. 2 Examples of Natural and Synthetic scaffolds