

Biological and synthetic scaffold: an extra cellular matrix for constructive tissue engineering

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Abstract

Worldwide many people suffering from tissue dysfunctions or damages need rapid transplantation. Tissue engineering has attracted attention as therapeutic modality aiming at repairing lost or damaged tissues. Critical step in tissue engineering is fabrication of three dimensional scaffolds which mimic the extracellular matrix of tissues and promote tissue regeneration process. Extensive research has been carried out to develop a compatible scaffold which mimic the anatomical site of injury and as well as accessing the stem cells and growth factors to home on the injured site. The present article provides an overview on different scaffold approaches and materials used to fabricate scaffolds, with their properties and associated advantages and disadvantages. In particular, the therapeutic potential of amniotic membrane and collagen scaffold has been extensively reviewed in here.

Key words: Amniotic membrane, Biological scaffold, Collagen, Synthetic scaffold, Tissue engineering

Introduction

Organ transplantation- the conventional treatment for tissue defects occurred due to disease, trauma, accident or aging include transplantation of tissue from one site to other from the same individual (autograft) or from other individual (allograft) or from other species (xenograft). Autografts have the problems of donor site morbidity and painful harvesting procedure, while allografts suffer from the immune rejection and dearth of organ donor, highlighting the need for new therapeutic modalities. Tissue engineering and regenerative medicine have come up as expanding approach to overcome the associated limitations with

classical approach that aim to repair, reconstruct or improve the function of damaged or diseased tissues with the use of combination of cells, materials and engineering methods. The important triad components in tissue engineering are cells, growth factors and scaffolds. Scaffold is the key component and its function is to act as support on which cells can adhere, proliferate and guide the regeneration of lost tissues. When designing and selecting a scaffold for tissue engineering, there are number of key points to be considered [1,2].

Biocompatibility: The very first criterion for any scaffold to be used in tissue engineering is biocompatibility, that means scaffold should not elicit any responses immunological and histological, and should be easily accepted by body.

Biomimetic: The scaffold should mimic the extracellular matrix of surrounding environment in terms of composition as well architecture where it has going to be implanted. The scaffold should possess cell adhesion sites; so cells can adhere and proliferate well on scaffold.

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Biodegradable: The scaffold should be degradable at the rate of new tissue formation obviating the need of scaffold removal. Biodegradability of scaffold allows cells to develop their own extra cellular matrix and repair tissue defects. Furthermore, the byproducts of scaffold degradation also should not be toxic.

Mechanical property: The scaffold should possess mechanical properties similar to implantation sites which protect cells from damaging compressive or tensile forces. The scaffold should have mechanical integrity from the time of implantation to the remodeling process.

Architecture: The architecture of scaffold greatly influences the cellular adhesion and proliferation on scaffold. The scaffold should possess porous structure to facilitate cell infiltration, new tissue formation, nutrients and metabolite transport and gaseous exchange.

Scaffold approaches in tissue engineering: Two major approaches have been evolved in tissue engineering for scaffold fabrication. One is synthetic scaffold in which scaffolds are fabricated using biomaterials. Another is biologic scaffold fabricated with tissues either from allogenic or xenogenic source. Before discussing these approaches in detail, Table 1 highlights the principle and advantages and disadvantages associated with both approaches.

Table-1: Characteristics of synthetic and biologic scaffold approaches.

Scaffold approach	Synthetic scaffold	Biologic scaffold
Material choice	Diversified choice of materials; different kinds of materials- polymers, ceramic or metal can be composited together	Allogenic or xenogenic biological tissue
Fabrication Technology	A number of fabrication techniques available	Decellularization process
Large scale production	Possible	Depends on availability of tissue source
Biomimetic	Materials and technique selection are critical steps to make biomimetic scaffold. Different kind of materials are composited together or cell adhesion peptide sequence are chemically added to make biomimetic scaffold	Nature simulating scaffold
Immunogenic	Metals, synthetic polymers and their degradation by products can be immunogenic	Decellularization process removes xenogenic and allogenic antigen
Disease transmission risk	Scaffolds fabricated with raw chemical materials; rare chance of any bacterial and viral infections	Derived from natural source, so there is risk of disease transmission
Application	Can be used for both soft and hard tissue application	Can be used for tissues with high extracellular matrix (ECM) content and for soft tissue

Synthetic scaffold: The synthetic scaffold approach employs biomaterials to fabricate scaffolds. Key steps in fabrication of synthetic scaffolds are selecting the material and technology that has led to enormous research in developing novel biomaterials and fabrication technology. Various scaffold fabrication technology such as phase separation, porogen leaching, electrospinning, rapid prototyping, injection molding etc. have been developed [3]. In this review we are not concentrating on these technologies and only focusing on properties and different kind of materials used for scaffolding.

Biomaterial is defined as natural or synthetic material that is suitable for introduction into living tissue especially as part of medical device. Enormous choices are available, when selecting the scaffold material. Biomaterials used for scaffold fabrication can be mainly categorized into metals, ceramics, natural and synthetic polymers. Each of these biomaterial groups has certain properties that can be tailored for a particular application [4].

Metals: Metallic scaffolds have find applications particularly in load bearing tissues because of their high fatigue resistance and compressive strength. The limitations associated with metals are- poor bioactivity, non-degradable in nature, release of toxic metals.

Ceramics: Ceramics are investigated in different forms such as powdered or granular form, injectable form or as coating on prosthesis. Hydroxyapatite (HA) is one of the widely explored ceramic because of its similarity to mineral phase of bone and osteoconductive nature. Hydroxyapatite containing scaffolds also enhances the differentiation of mesenchymal stem cells towards osteogenic lineage. Ceramics possess advantages of high mechanical stiffness, low elasticity but their brittleness and difficulty in shaping limit their application alone.

Synthetic polymers: A number of synthetic polymers including polycaprolactone (PCL), poly l-lactic acid (PLLA), poly-lactic- glycolic acid (PLGA), polystyrene, polyglycolic acid (PGA), poly vinyl alcohol (PVA), polyurethane etc. have been explored in different tissue engineering applications. Associated advantages with synthetic polymers are reproducibility, large scale production, tailored mechanical properties, and disadvantages are lack of cell recognition sites, and risk of rejection. The degradation of synthetic polymers is also a concerning issue, as acidic hydrolysis of these polymers cause reduction in pH of surrounding environment.

Natural polymers: Another class of materials that have attracted attention in artificial scaffold approach is natural polymers having the advantages of cell recognition sites and low antigenicity. Furthermore their biodegradable nature allows host cells to produce their own matrix. However natural polymers suffer from disadvantages like batch to batch variability and poor mechanical property. Table 2 provides glimpse of potential of various materials used in different tissue engineering applications.

Table-2: Different kinds of material explored in tissue engineering

Synthetic Material		Applications
Metals	Titanium	Orthopedic applications, Bone tissue engineering [5-7]
	Tantalum	Orthopedic application [8-9]
	Iron	Bone Tissue engineering [10-11]
	Magnesium	Bone Tissue engineering [12-13]
Ceramics	Hydroxyapatite	Bone tissue engineering [14-19]
	Tri calcium phosphate	Bone tissue engineering [20-22]
	Bioactive glass	Bone tissue engineering [23-24]
Synthetic polymers	Poly caprolactone (PCL)	Bone, skin , vascular tissue engineering, drug delivery [25-30]
	Poly glycolic acid (PGA)	Surgical sutures, bone, skin, vascular tissue engineering [31-34]
	Poly lactic-glycolic acid (PLGA)	Bone, cartilage repair, skin, vascular tissue engineering [35-41]
	Polyurethane	Bone, cartilage repair, skin , vascular tissue engineering [42-47]
Natural Polymer	Collagen	Skin, bone, cartilage, blood vessel tissue engineering [48-53]
	Gelatin	Skin, bone, cartilage, blood vessel, stem cell delivery [54-59]
	Alginate	Drug delivery, Skin, liver tissue engineering [60-62]
	Chitosan	Wound healing, skin, bone, cartilage tissue engineering [63-68]
	Fibrin	Wound healing, skin ,bone, vascular tissue engineering [69-74]

Case Study- Collagen scaffold

Properties: The most abundant protein in extracellular matrix is collagen and is present in various tissues including blood vessel, bone, cartilage, tendon, ligament, skin etc. Collagen is stable macromolecule comprised of three polypeptide chain woven into triple helix. Major roles of collagen are to maintain the structural integrity of tissues and

regulate adhesion, migration and proliferation of cells. Collagen possesses poor immunogenic property in comparison to other proteins. The presence of cell recognition peptide sequences on collagen allows cellular adherence and proliferation. Collagen is easily biodegradable in the presence of collagenase enzyme and their degradation profile can be controlled by crosslinking collagen. Low immunogenicity, biocompatibility and biodegradability make it widely explored polymer in tissue engineering and regenerative medicine.

Therapeutic application of Collagen scaffold: Collagen scaffolds can be fabricated in different forms such as thin sheets, sponges, and hydrogels with employing different techniques. To enhance their mechanical stability, collagen scaffold has been prepared by combining it with other polymers and ceramics such as chitosan, gelatin, PCL, PLLA, hydroxyapatite. A large number of *in-vitro* as well as animal studies have been done with collagen scaffold for various tissue engineering applications, though only some clinical studies are reported with collagen scaffold. Clinical studies done with collagen scaffold have been summarized in Table 3.

Table-3: Therapeutic application of Collagen scaffold: Clinical trials.

Therapeutic application	Collagen scaffold	Author	Results
Eye	Carbodiimide crosslinked recombinant human collagen	Fagerholm P <i>et al</i> (2014)	Patients grafted with RHC implants had a 4-year average corrected visual acuity of 20/54 and gained more than 5 Snellen lines of vision on an eye chart [75].
Nerve graft material	Collagen matrix tubes	Ashley WW Jr <i>et al</i> (2006)	Four of the five patients experienced a good recovery, and three exhibited an excellent recovery at 2 years postoperatively. The Motor scale composite was improved by an average of 69 and 78% at 1 and 2 years respectively. No complications were seen [76].
Nerve repairs in the forearm	Type 1 collagen nerve conduits	Dienstknecht T <i>et al</i> (2013)	No implant-related complications were observed. Out of 9 patients, 8 patients were satisfied. Collagen conduits can be an efficacious method for repairing nerves in forearm [77].
Lingual and inferior alveolar nerve injuries	Bioabsorbable collagen nerve cuff	Farole A <i>et al</i> (2008)	8 out of 9 nerve repairs exhibited sensory improvement suggesting role of NeuraGen as a nerve cuff and protective barrier around the nerve injury site [78].
Digital nerve lacerations	Collagen conduit	Taras JJ <i>et al</i> (2011)	Nerve lacerations in 19 patients were reconstructed with a bioabsorbable collagen conduit. All patients recovered protective sensation [79].
Endodontics	Collagen scaffold	Sharma S <i>et al</i> (2016)	Platelet rich fibrin and collagen exhibited better results than blood clot and PLGA in measurement of periapical healing, apical closure, and dentinal wall thickening [80].
Cartilage defects	Atelocollagen gel	Ochi M <i>et al</i> (2002)	Autologous chondrocytes, cultured in atelocollagen gel were transplanted to patients having full-thickness defects of cartilage. Transplantation eliminated locking of the knee and reduced pain and swelling in all patients [81].
Burns and Chronic wounds	Collagen dressing	Singh O <i>et al</i> (2011)	Collagen dressed wound exhibited healthy granulation tissue than conventionally treated wounds (P=0.03). Collagen-treated patients had early and more subjective mobility [82].

Biological scaffold: Biological scaffold approach employs the scaffolds derived from biological tissues such as extracellular matrix and amniotic membrane. The ECM is derived from various tissues such as blood vessels, skin, nerves, tendon, small intestinal submucosa and amniotic membrane is obtained from placenta during delivery.

Extracellular matrix scaffold consists of complex molecules secreted from resident cells of tissues that are arranged in tissue specific unique 3D structure. The composition of ECM depends on the tissue it has been derived but overall it is a rich source of proteins, proteoglycans and various growth factors. As a scaffold for tissue engineering, ECM not only provides structural support to cells and tissues, but also has growth and signaling factors having angiogenic, chemotactic, antimicrobial properties. Decellularization and sterilization techniques have been developed to produce decellularized low immunogenic sterile ECM scaffolds. Decellularization mainly comprised of mechanical and enzymatic techniques, which remove the xenogenic and allogenic cellular components from the tissues, without compromising the architecture and components of ECM. Extra cellular matrix derived scaffolds have been utilized for different tissue engineering applications including musculoskeleton, cardiovascular, skin tissue engineering etc [83-91].

Case study-Amniotic Membrane (AM): Amniotic membrane derived from placenta possesses a lot of inherent properties which makes it a suitable candidate to be explored as scaffold. The amniotic membrane usage has started since early 20th century, but the advancement in preservation and processing techniques of amniotic membrane has extended its usage largely in last 10 years in reconstructive medicines. The following section deals with properties and therapeutic potential of amniotic membrane in detail.

Anatomy of Amniotic membrane: Amniotic membrane is the innermost thin membrane of placenta which protects the fetus from surrounding environment. The thickness of amniotic membrane varies from 0.02-0.05mm. On microscopic examination, the amniotic membrane consists of three layers- epithelium layer, basement membrane and a vascular stroma. The innermost layer is epithelial layer consisting of single layered of cells arranged on basement membrane. The amniotic membrane derives its nutrition by diffusion process through amniotic fluid, because it does not contain any blood vessels or nerves. With material point of view, amniotic membrane contains three kind of materials- extracellular matrix, cells and molecules. The components which make the architecture of membrane contains-collagen I, III IV, V and VII, hyaluronic, fibronectin, proteoglycans, laminin etc. Majorly two types of cells are present in amniotic membrane- amniotic epithelial cells and amniotic mesenchymal stem cells. The important biomolecules present in AM are fibroblast growth factor, platelet derived growth factor, transforming growth factor-beta, and metalloproteinases [92].

Properties: The amniotic membrane possesses several inherent biological properties which make it a potential candidate as scaffold for various therapeutic potential.

Anti-inflammatory: Several reports available in literature exhibit anti-inflammatory property of amniotic membrane. In a study done by Shimmura *et al*, monocyte and macrophage cells infiltration were observed in amniotic membrane patches after one week of appliance to ocular surface with corneal epithelial defects [93]. The reduction in inflammation was reported with topical application of culture supernatant from human amniotic epithelial cells to dogs via inhibiting the IL-beta and nitric oxide (NO) production [94].

The anti-inflammatory action is also possessed in amniotic membrane extract also. Various anti-inflammatory and anti angiogenic proteins in amniotic epithelial cells as well in amniotic membrane stroma have been identified by Hao *et al* [95]. He *et al*. purified a covalent linked complex of heavy chain of inter alpha inhibitor (HC.HA) with abundant hyaluronan (HA) which is responsible for anti-inflammatory action [96].

Non immunogenic: Non immunogenicity is one of the important properties, the scaffold should possess for transplantation. The amniotic epithelial cells do not express many major histocompatible complexes like human leukocyte antigen (HLA)-A, -B and -DR antigens, while express HLA-G conferring immuno-privileged status to amniotic graft. The function of HLA-G is to induce immune tolerance by acting as ligand for inhibitory receptors present on macrophages. Cryopreserved amniotic membrane is known to possess low immunogenicity in comparison to fresh amniotic membrane due to non-viability of cells on cryopreserved membrane [97].

Anti-scarring property: Amniotic membrane possesses anti-scarring action by suppressing transforming growth factor (TGF)-beta signaling pathway [98]. In a study performed by Tseng *et al*, amniotic membrane matrix reduced expression of transforming growth factor beta isoforms in cultured human corneal and limbal fibroblasts [99].

Anti-angiogenic property: In addition to anti-inflammatory and anti-scarring properties, amniotic membrane also possesses antiangiogenic action. Anti-angiogenic compounds such as endostatin, tissue inhibitor metalloproteases have been identified in amniotic membrane. Delay in graft vascularization occurred with AM transplant after pterygium surgery in comparison to conjunctival autograft.

Studies exhibited the angiogenic action of amniotic membrane also [100]. Amniotic membrane has side dependent angiogenic and anti-angiogenic property increase in angiogenesis was observed in mesenchymal side up, while decrease in angiogenesis was exhibited in epithelial side up [101].

Antimicrobial activity: One of the important properties of amniotic membrane is its antimicrobial activity. During pregnancy, amniotic membrane's antimicrobial action protects the fetus from any bacterial and fungal infection. The amniotic membrane expresses β defensins- antimicrobial peptide, elastase inhibitor, leucocyte proteinase inhibitor, which are component of innate immune system.

Various studies have reported the antimicrobial action of amniotic membrane against many Gram positive and Gram negative bacteria [102-103]. The antimicrobial activity of amniotic membrane is retained even after cryopreservation and freeze drying process [104].

Therapeutic applications of Amniotic membrane:

Ophthalmology: Since 60 years, amniotic membrane transplantation is being used in ophthalmology. There are two major modes to transplant amniotic membrane- either it can be applied as permanent graft in which it act as substrate for cells to grow or temporary bandage or patch in which it act as covering. Amniotic membrane has been used successfully as surgical graft for wide range of ophthalmic conditions (Table 4).

Amniotic membrane in wound healing: Amniotic membrane has gained much popularity in wound and burns treatments because of its ability to reduce scarring, inflammation and enhance epithelialization and wound healing. Amniotic membrane has found wide application in treating different kinds of wounds including diabetic foot ulcer, varicose ulcer, venous leg ulcer, neuropathic foot ulcers etc [120-125].

Various clinical studies done with amniotic membrane for treatment of different kind of wounds have been summarized in our earlier publication [126].

Other therapeutic application of Amniotic membrane: Various clinical studies have been done with amniotic membrane in periodontics also. First in 1997, Gular *et al*. studied the use of amniotic membrane for vestibuloplasty in 20 patients [127]. In initial days, patients exhibited edema, higher blood flow, but with time grafted area was completely covered and blood flow was also normal.

The efficacy of amniotic membrane for ridge preservation following tooth extraction was studied by Wallace *et al* and reported no inflammation and excellent bone quality formed [128]. In pre-malignant lesion leucoplakia, the left buccal mucosa was covered with amniotic membrane graft and defect was restored with out any complications.

In periodontics, amniotic membrane graft has shown its efficiency in treating gingival recession, periodontal intrabony defects [129-132].

Amniotic membrane has also been explored recently for other therapeutic applications such as in cartilage restoration, tendon healing, osteoarthritis, planar fasciitis [133-140].

Table-4: Clinical application of Amniotic membrane in different Ophthalmic Conditions

Disease	Authors	Results
Infectious Keratitis	Gicquell JJ <i>et al</i> (2007)	Amniotic membrane transplantation combined with topical corticosteroid promoted epithelial healing and reduced pain in severe bacterial keratitis [105].
Infectious Keratitis	Kim JS (2001)	In infectious corneal ulcer, amniotic membrane transplantation promoted wound healing and reduced inflammation [106].
Infectious Keratitis	Sheha H <i>et al</i> (2010)	The transplantation of amniotic membrane actively promoted wound healing in managing severe infectious keratitis [107].
Cornea Ulceration	Hanada K <i>et al</i> (2001)	Multilayered amniotic membrane was effective method for treatment of deep ulcers of cornea and sclera [108].
Corneal perforation, ulcers	Soloman A <i>et al</i> (2002)	AM transplantation was an effective method for non traumatic corneal perforations [109].
Corneal perforation	Rodriguez Aries MT <i>et al</i> (2004)	Multilayer AM was found effective in treating corneal perforation [110].
Cornea Epithelial defect	Prabhasawant P <i>et al</i> (2001)	AM successfully treated corneal epithelial defect by promoting epithelial healing and preventing corneal perforations. No graft rejection was observed [111].
Cornea Epithelial defect	Seitz B <i>et al</i> (2009)	AM transplantation was beneficial for treating persistent epithelial defects, when applied in sandwich method [112].
Bullous keratopathy	Stefaniu GL <i>et al</i> (2014)	AM transplantation was efficient in treating oedematous keratopathy. In 88% of cases, improvement was observed [113].
Bullous keratopathy	Mrukwa-Kominek E <i>et al.</i> (2002)	AM transplantation was beneficial in the process of corneal healing and improved visual activity [114].
Limbal stem cell deficiency	Anderson <i>et al</i> (2001)	AM transplantation was effective to restore stable corneal epithelium with partial limbal stem cell deficiency and can be an alternative to limbal autograft and allograft [115].
Limbal stem cell deficiency	Gomes <i>et al</i> (2003)	AM transplantation was efficient for ocular surface reconstruction in chemical burns having limbal stem cell deficiency [116].
Pterygium surgery	Katbaab <i>et al</i> (2008)	AM transplantation is safe and effective method in primary pterygium surgery with low recurrence rate [117].
Conjunctivochalasis	Meller D <i>et al</i> (2000)	Defects were healed in 16.5 +/- 7.3 days. Episodic epiphora was resolved in 24 of 30 (83.3%) eyes [118].
Conjunctivochalasis	Georgiadis NS <i>et al</i> (2001)	No patient had complain of epiphora and no conjunctivochalasis was detected in the area in which human amniotic membrane was transplanted [119].

Conclusion

Critical steps in tissue engineering are selection of material and technology to fabricate scaffold. The main objective of all scaffold fabrication technique is to fabricate scaffold with materials which can mimic the extracellular matrix of targeted tissue as close as

possible. The present article has reviewed the properties and therapeutic potential of biological as well as synthetic scaffolds. A wide range of materials including natural, synthetic, ceramic, metals, biological and their composites can be fabricated as scaffold in tissue

engineering and regenerative medicines and there is continuous research going on to enumerate their full potential. Still state-of-the-art synthetic scaffolds has to undergo clinical trials and there is a long way to go from bench to bedside.

However the advancements in processing and preservation technology have enhanced the popularity of biologic scaffolds as graft in various tissue engineering applications. Amniotic membrane provides many advantages over synthetic scaffolds firstly it is available in ample amount at low cost and processing is also very simple. The preservation procedures allow it to store for longer time and use it when required. These preservation procedures also remove the risk of any infection transmission. Furthermore, amniotic membrane being natural material gets easy acceptance from host and there are no reports of graft rejection with amniotic membrane. In addition to biocompatibility, it is permeable, stable, flexible and resorbable with time. Published literature exhibits the wide usage of amniotic membrane in ophthalmology and wound healing and continued to be explored in periodontics, cartilage, tendon etc. However the use of amniotic membrane scaffold for all applications is not possible especially for load bearing application. Further studies are needed to be performed with biological and synthetic scaffolds and their composites to have optimized scaffolds that imitate biological tissues in terms of both structure and function.

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