

Biomimetic Scaffolds for Skin and Skeletal Tissue Engineering

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Regenerative medicine is a vastly growing field that aims to restore biological functions of damaged tissues or organs. While autologous grafting remains the gold standard for treating skin and skeletal defects, complications from antigenicity, donor site morbidity, and limited donor tissue have spurred the development of tissue engineering for regenerative therapies [1,2]. Mimicking the 3D structure and extracellular matrix (ECM) of native tissue is critical for successful cell transplantation and growth of artificial tissue [3]. Biomimetic scaffolds are therefore necessary to recapitulate this natural environment and provide various cues to direct cell processes and differentiation [4,5]. The ideal scaffold material must have high biocompatibility, tunable biodegradability, and nontoxic by-products [6,7]. The structure must also have adequate surface area for cell adhesion as well as macroporosity for cell migration and proliferation [7,8]. The ideal scaffold will also have bioactive characteristics, such as mechanical or chemical properties, to direct cells towards specific tissue phenotypes [5]. In this review, we discuss advances in scaffold technology for tissue engineering and highlight current trends in scaffold materials used (Table 1).

Collagen

Collagen is a natural polymer and constitutes a substantial component of skin, cartilage, and bone [9]. Although there are 28 known variations of this protein in the collagen superfamily, type I collagen is the most abundant in the human body and is commonly used for scaffold synthesis [10]. Due to its excellent biocompatibility, degradability, and abundance in the body, collagen is highly desirable as a scaffold material in tissue engineering. In addition, collagen binds with most integrins for enhanced cell adhesion [7]. Hydrogels made of collagen are cross-linkable and often injectable for promoting wound repair, and commercial products like Excollagen[®], synthesized from type I collagen, are used in diabetic foot ulcer treatment to promote reepithelialization and granulation tissue growth [7,11]. Although collagen mimics many of the natural ECM's properties and structures, hydrogels often lack the ideal three-dimensional (3D) and mechanical properties necessary for different types of tissue [6,12]. Han et al. have reported a novel biomimetic, "microribbon-like" scaffold composed of type I collagen that has 3D structure, macroporosity for cell migration, and tunable stiffness [4]. This ability to mimic biomechanical cues of various tissues is crucial for the engineering of specific cell lineages [8].

Hyaluronic Acid

Hyaluronic acid (HA) is an important glycosaminoglycan (GAG) present in synovial fluid and the ECM of connective tissue. Supraphysiological levels of HA have been shown to reduce inflammatory response as well as promote angiogenesis, making it an ideal material for regenerative therapies [13,14]. HA also has good biocompatibility and biodegradability which triggers minimal immune response [9,15]. Studies have shown that HA scaffolds can successfully promote healing of both cartilage and skin defects due to their bioactive

properties [16]. HYALONECT[®] (Anika Therapeutics, USA) is one HA-based mesh sold commercially outside of the United States that has been shown to promote bone regeneration and restore periosteum function in orthopaedic defects [17].

Fibrin

Fibrin is a glycoprotein responsible for blood clots and hemostasis during injury. Products such as QuikClot[®] (Z-Medica, USA) and Fibrin Sealant Dressing have both utilized this quality to prevent blood loss during combat and emergency procedures [18]. Fibrin gels are also promising as a scaffold since they naturally act as a provisional ECM that can sequester growth factors or specific cell types [19]. They are particularly desirable as well since they can be synthesized from autologous blood to reduce immunoresponse and disease transmission while maintaining biocompatibility [9]. Johnson et al. have shown that fibrin scaffolds can increase cell proliferation and migration for treatment of spinal cord injuries via growth of neural fibers [20]. Other studies have shown its promise as a minimally-invasive delivery method for mesenchymal stem cells (MSCs) into bone defects to promote osteogenesis [21].

Chitosan

Chitosan, derived from the exoskeleton of insects and crustaceans using N-deacetylation, is a polysaccharide with cell-adhesive qualities [5,22]. Scaffolds made from chitosan are highly porous, 3D, and have structural resemblance to many critical GAGs found in cartilage [23]. In addition, chitosan has intrinsic antibiotic qualities and can be used to prevent infections of bone [24]. It is biocompatible and biodegradable by lysozymes as well, and chitosan scaffolds have been used for cartilage, bone, and skin treatment [9]. Suh et al. have demonstrated that a chitosan-based membrane influences seeded cells towards a chondrocyte morphology and may be an ideal scaffold for cartilage tissue engineering [25].

Decellularized ECM

ECM-derived scaffolds are synthesized from animal tissue by removing the host cells while leaving behind the necessary proteins,

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Scaffold Material	Biomimetic Properties	Examples	Tissue Engineering Applications
Collagen	- Natural polymer, found in skin, cartilage, and bone - Integrin binding sites for enhanced cell adhesion - Biodegradable and non-toxic	- Excellagen® - Hydrogels - Microribbons	- Cross-linkable and injectable for wound repair - Promote reepithelialization and granulation tissue growth in diabetic ulcers - Tunable for tissue-specific cues
Hyaluronic Acid	- GAG found in synovial fluid and connective tissue ECM - Excellent biocompatibility and biodegradability	- Hydrogels - HYALONECT®	- Inherently reduces inflammatory response and promotes angiogenesis in tissues - HA-based mesh promotes bone and periosteum regeneration
Fibrin	- Glycoprotein responsible for hemostasis during injury - Natural provisional ECM to sequester growth factors and cells	- QuikClot® - Fibrant Sealant Dressing - Hydrogels	- Made from autologous blood to prevent immunoresponse - Minimally-invasive delivery method for MSCs into bone defects
Chitosan	- Naturally occurring polysaccharide with cell-adhesive qualities - Structural resemblance to many crucial GAGs in cartilage	- Hydrogels - Antibiotic-loaded bar	- High porosity, 3D structure, and GAG content ideal for chondrogenesis - Intrinsic antibiotic qualities to prevent infection of tissue
Decellularized ECM	- Contains crucial proteins, GAGs, and structural components - Biocompatible and biodegradable	- GraftJacket®	- <i>In vitro</i> engineering of scaffolds for autologous applications - Improve healing of chronic diabetic foot ulcers

Table 1: Biomimetic scaffolds for skin and skeletal tissue engineering.

GAGs, and structural components critical for tissue engineering [7]. While previously believed to be inert, the ECM actually has a complex and influential relationship with cells through its various binding sites and growth factors [26]. Decellularized ECM scaffolds they are therefore ideal for mimicking native host tissue properties. Since it derived from natural tissue, these scaffolds are highly biocompatible and biodegradable [9]. However, removing cellular artifacts that illicit immunoresponse while perfectly maintaining the intrinsic ECM components has proven challenging [27]. Recent studies, however, have demonstrated the potential for engineering autologous, ECM-derived scaffolds through *in vitro* expansion and subsequent decellularization of a subject's own cells to promote tissue growth [28]. GraftJacket® (Wright Medical Technology, USA) is one commercial product derived from human dermis that has been clinically shown to improve healing of chronic diabetic foot ulcers [29].

Future Directions: Therapeutic Delivery

While mimicking the natural ECM and structure of the native milieu is important for tissue engineering, a novel approach is to incorporate various growth factors and signaling molecules into scaffolds for controlled release [26]. Growth factors such as bone morphogenic proteins (BMP_s), transforming growth factor beta (TGF-β), and basic fibroblast growth factor (bFGF) have proven critical for proper wound repair and tissue regeneration [30]. Fujisato et al. demonstrated enhanced chondrocyte proliferation and maturation using a collagen sponge scaffold loaded with bFGF [31]. Sun et al. used a TGF-β3 lentiviral vector loaded onto a scaffold seeded with MSCs to promote chondrogenesis [32]. However, using supraphysiological levels of these factors can have negative side effects. The medical device INFUSE® (Medtronic, USA) was approved to induce spinal fusion by releasing recombinant human BMP-2 at concentrations a million times higher than normal, but many complications such as bone overgrowth and cervical swelling have since been reported [7]. To mitigate the negative side effects associated with these supraphysiological concentrations, another strategy is to utilize microspheres containing growth factors for controlled release *in vivo* [33]. Fan et al. developed such microspheres loaded with TGF-β1 and seeded them onto a collagen-GAG scaffold to promote differentiation of MSCs into chondrocytes *in vivo* [34].

Conclusion

While the perfect biomimetic scaffold does not currently

exist, recent advances in tissue engineering have resulted in more commercially available products and promising new therapies. As research in regenerative medicine continues to grow, we anticipate novel scaffold technology, including but not limited to, autologous “off the shelf” cell-based scaffolds and incorporation of combination strategies involving cells, gene therapy, small molecules, and mechanical forces. With continued research, improved scaffolds will pave the way for advancements in regenerative medicine.

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