Review Article

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Advances in biomaterials for adipose tissue reconstruction in plastic surgery

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Abstract: Adipose tissue reconstruction is an important technique for soft tissue defects caused by facial plastic surgery and trauma. Adipose tissue reconstruction can be repaired by fat transplantation and biomaterial filling, but there are some problems in fat transplantation, such as second operation and limited resources. The application of advanced artificial biomaterials is a promising strategy. In this paper, injectable biomaterials and three-dimensional (3D) tissue-engineered scaffold materials for adipose tissue reconstruction in plastic surgery are reviewed. Injectable biomaterials include natural biomaterials and artificial biomaterials, which generally have problems such as high absorptivity of fillers, repeated injection, and rejection. In recent years, the technology of new 3D tissue-engineering scaffold materials with adipose-derived stem cells (ADSCs) and porous scaffold as the core has made good progress in fat reconstruction, which is expected to solve the current problem of clinical adipose tissue reconstruction, and various biomaterials preparation technology and transformation research also provide the basis for clinical transformation of fat tissue reconstruction.

Keywords: adipose tissue reconstruction, biomaterial, plastic surgery

1 Introduction

Adipose tissue reconstruction is an plastic surgery aimed at achieving volume recovery and adipose tissue regeneration [1,2]. The surgery is not only used for routine cosmetic treatment, such as wrinkle removal, but also widely used for congenital defects, trauma, or reconstruction of surgically removed tissues, such as breast collapse after breast tumor surgery [3] and soft tissue deficiency after maxillofacial surgery [4]. Adipose tissue reconstruction usually uses the transfer of autologous adipose tissue or the construction of biomaterials suitable for filling requirements. Adipose tissue metastasis has the advantages of low immune rejection and light inflammatory response, which has been introduced into daily clinical treatment [5]. However, autologous fat transplantation is accompanied by inevitable fat absorption and donor region complications [6]. So, researchers are turning to biomaterials. Biomaterials are inanimate materials used for medical purposes to contact with living tissues [7]. Common implantable biomaterials include hyaluronic acid (HA), collagen, and polymethyl methacrylate (PMMA). Compared with autologous fat transplantation, biomaterials have the advantages of easy access, no damage to the donor area and modification according to requirements, but also have the disadvantages of foreign body reaction and inflammation, deformation, and repeated injection due to absorption of filler materials [7–10]. Although fat transplantation and biomaterials have been widely used in clinical practice, problems such as multiple operations because of material absorption and rejection reactions are not completely solved. The idea of three-dimensional (3D) biological scaffolds is to construct extracellular matrix (ECM) scaffolds

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and combine them with adipose-derived stem cells (ADSCs) and other cells, so as to provide an environment for the differentiation and growth of adipocytes and ultimately achieve the reconstruction of adipose tissue [11]. Now many studies of the combination of injectable biomaterials and biomaterial stents with fat transplantation show great potential in preclinical studies. This paper reviews the recent research advances in biomaterials for surgery of adipose tissue reconstruction.

2 Injectable biomaterials for adipose tissue reconstruction in plastic surgery

The materials used for adipose tissue reconstruction are generally injectable hydrogel materials because they can be simply injected subcutaneously, greatly avoiding pain and complications in patients. In addition, the injected biomaterials can fill the complex lacuna in the patient's surgical area, avoiding the formation of dead space [12]. Therefore, injectable biomaterials are widely used in adipose tissue reconstruction. Injectable *in situ* gels can be prepared by various physical and chemical methods. Physical methods include temperature, pH, electric and magnetic fields, enzymatic method, optical method, and hydrophobic interaction, etc., while chemical methods include various crosslinking methods, such as Schiff crosslinking method, Michael crosslinking method, and enzyme-catalyzed crosslinking method [5,7,13]. The preparation of various injectable biomaterials is complicated, but the materials can be divided into natural materials and synthetic materials. The basic research and clinical application progress of various injectable biomaterials are introduced from natural biomaterials and synthetic biomaterials, respectively.

2.1 Natural biological materials

2.1.1 Collagen

Collagen is the main component of connective tissues, ligaments, and tendons, and collagen in the natural ECM is an important biomaterial [14,15]. Currently, there are three main sources of collagen biomaterials. The first is bovine collagen extracted from bovine tendons, dermis, and bones [16]. Bovine collagen can form a firm structure through collagen fibers after injection to help correct facial defects. Now various bovine collagen products have been on the market for correcting scars, crow's feet, and periorbital wrinkles [5,17]. However, it has been reported that bovine collagen has a high incidence of rejection [18]. The second is pig collagen, which is extracted from the tendons, dermis, and bones of pigs. Recently, materials using pig collagen have been promoted as intraepithelial fillers. Compared with bovine collagen, pig collagen has higher wrinkle resistance and no serious adverse reactions [19]. The third is human collagen material extracted from the dermis of the human body. It can be extracted from adipose tissue after liposuction or produced by recombining yeast or bacteria of human collagen [20,21]. Human collagen material has been used in clinical practice, delaying the degradation of collagen lysine residues by cross-linking them with glutaraldehyde, mainly for deep scars and wrinkles [20]. The human collagen material has shown good clinical effect, but the price is expensive, and the long-term effect is yet to be verified by clinical studies (Table 1).

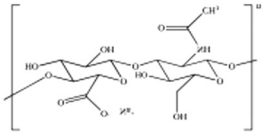
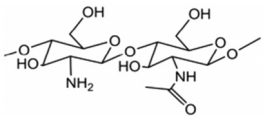
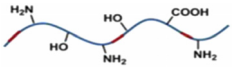
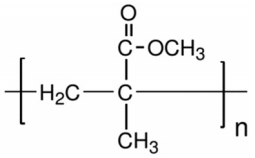
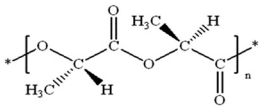
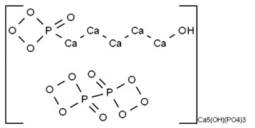
2.1.2 HA

HA is a naturally occurring glycosaminoglycan that is usually extracted from bacteria or the cockscomb of roosters. HA has shown low immunogenicity in preclinical studies, and several HA materials have been approved for clinical use [22,23]. However, HA degrades quickly and requires repeated injections and lacks the ability to induce adipose cells, so it is usually used as a temporary filler to induce tissue repair [24]. Several studies have focused on reducing the degradation rate of HA to ensure its volume persistence and improve its biological performance. At present, there are two main methods to degrade the degradation rate of HA, one is the double-crosslinking method [24] and the other is the combination of HA with biopolymers such as gelatin, chitosan, cellulose, or synthetic polymers such as polyethylene glycol (PEG) [25–27]. Compared with pure HA, the combination of HA and gelatin shows good biocompatibility and lower degradation rate. Although the anaphylaxis caused by HA is weak, there are still pain, bruising, and temporary edema in the local area which have been reported [28].

2.1.3 Chitosan

Chitosan is a natural linear polysaccharide obtained by partial deacetylation of chitin. In tissue engineering applications, its water-soluble analog N-SCS is often used as a tissue filler [29]. Tan et al. [30] developed a biodegradable hydrogel construct composed of succinyl chitosan (SCS), PEG, and insulin, in which insulin acts as

Table 1: Advanced biomaterials for adipose tissue regeneration and their applications

	Name	Molecular structure/formula	Resource	Advantages	Disadvantages	Application
Natural biomaterials	Collagen [5,14–21]	None	Bovine collagen, pig collagen and human collagen	Superior biocompatibility	Rejection reaction; possible disease transfection	Facial soft tissue filler for crow's feet, periorbital wrinkles, and deep scars
	Hyaluronic acid [22–28]		Bacteria or the cockscomb of roosters	Minimal rejection reaction	Mild swelling; rapid degradation	Temporary filler to induce tissue repair
	Chitosan [29,30,68]		Partial deacetylation of chitin	Nontoxic; slowly biodegradable; biocompatible	Rejection reaction; limited promotion of cell proliferation	Soft tissue filler; wound healing
	Gelatin [31,32,69]		Partial hydrolysis of collagen	A good vehicle for adipose tissue engineering	Poor mechanical properties	Biological scaffold for adipose tissue engineering
	Decellularized adipose tissue [33–35]	None	Adipose tissue	Potential benefits for repairing of damaged tissues	Rapid degradation; repeated injection	Biological scaffold for adipose tissue engineering; wound healing cartilage and bone regeneration, nerve injury repairing
Synthetic biomaterials	PMMA [36,37]		Artificially synthesized	Long-lasting	Rejection reaction; nonbiodegradable	Volume enhancer and profiler for nasolabial line, radial upper lip line, interbrow line and corner line
	PLLA [38–41]		Artificially synthesized	Superior biocompatibility; provoke collagen deposition	Rapid biodegradation	Soft tissue reconstruction; biological stimulant
	Calcium hydroxyapatite [42,43]		Artificially synthesized	Long-lasting; biocompatibility; biodegradability; low toxicity	Rejection reaction	Nasolabial folds

an adipogenic factor. When the hydrogel was cultured with cells for 6 h, the adhesion of cells to SCS/PEG hydrogel was significantly greater than that to pure SCS hydrogel (the relative DNA content increased by about 1.5 times).

2.1.4 Gelatin

Gelatin is a water-soluble biocompatible protein, which is the product of partial hydrolysis of collagen. It retains biological interaction signals including Arg-Gly-Asp (RGD) sequence, so it is often used in various tissue engineering applications. Tuin et al. [31] evaluated the potential of an injectable hydrogel composed of recombinant gelatin enriched by HA and RGD in rats. They found that after 4 weeks of transplantation, the introduction of recombinant gelatin induced soft tissue regeneration, characterized by significant vascularization and infiltration of fibroblasts and macrophages. Using transglutaminase to hydrolyze and cross-link gelatin hydrogel and encapsulating ADSCs, Zeno Alarake et al. [32] obtained good results *in vitro*.

2.1.5 Decellularized adipose tissue

Because decellularized adipose tissue (DAT) has the potential to enhance the regeneration and repair of damaged tissues, some studies have shown that ADSCs combined with DAT is an effective biofiller [33–35]. Zhang et al. [33] studied the potential of heparinized DAT loaded with basic fibroblast growth factor (bFGF) in mice. These injectable scaffolds induced significant adipogenesis and neovascularization within 6–12 weeks, whereas the non-crosslinked DAT (excluding bFGF) formed adipogenesis to a lesser extent and was almost completely absorbed after 12 weeks. Tan et al. [35] also observed that after subcutaneous injection of acellular pig fat tissue, fat generation was significantly enhanced after ADSC inoculation. Therefore, the application of DAT in combination with ADSCs is considered to be highly synergistic, as these systems demonstrate long-term lipogenesis and angiogenesis *in vivo*.

2.2 Synthetic biomaterials

2.2.1 PMMA

PMMA is a synthetic and nondegradable biocompatible polymer, which is often used as an intradermal filler in tissue repair. The PMMA microspheres developed originally are called Arteplast [36], because of their small molecular diameter (less than 20 μm), causing immune responses such as macrophage phagocytosis and inflammation. Artefill [37] is a new generation of developed PMMA microspheres, with a size of 30–50 μm, which is reported that it has a less risk of phagocytosis and can reduce the formation of granuloma. At present, it has been reported as a volume enhancer and profiler for nasolabial line, radial upper lip line, interbrow line, and corner line.

2.2.2 Poly-L-lactic acid

Originally synthesized from α-hydroxy acids, poly-L-lactic acid (PLLA) is a biodegradable thermoplastic polymer that has been widely used in orthopedics, neurosurgery, and suture materials of craniofacial surgery, absorbable plates, and screws [38]. The main use of PLLA in soft tissue reconstruction is to enlarge and fill the deep dermis or subcutaneous layer and repair facial fat atrophy [39]. PLLA expands at the moment of injection to fill and repair the lacuna and degrades after a period of time. PLLA can stimulate collagen production and vascularization in the whole process, which ultimately causes dermal fiber proliferation, acting as a biological stimulant [40]. Therefore, PLLA is also approved as a treatment for nasolabial fold, facial wrinkles, line, and contour enhancement defects [40,41].

2.2.3 Calcium hydroxyapatite

Calcium hydroxyapatite (CH) and its by-products naturally occurring in human bone and tooth enamel are composed of calcium and phosphate ions and have strong biocompatibility [42]. CH can be prepared as microspheres and suspended in carboxymethyl cellulose gel, which has been approved for the correction of facial wrinkles and the filling of soft tissues [43]. The CH microspheres in the gel can promote the formation of new tissues through collagen deposition. The gel usually degrades within 2–3 months and its by-products, calcium and phosphate ions, can be removed by phagocytosis of macrophages. Therefore, some studies believe that the treatment effect of CH on nasolabial folds is better than that of pure collagen materials [43].

3 Tissue engineering scaffold materials for adipose tissue reconstruction in plastic surgery

The application of 3D tissue-engineered scaffold materials for adipose tissue repair is an important method in the field

of adipose tissue reconstruction. The principle is to encapsulate adipose cells, ADSCs, or other cells and growth factors through tissue engineering, and then inoculate them on 3D scaffolds simulating ECM [11]. Good tissue engineering scaffolds in orthopedic adipose tissue reconstruction should meet the following requirements: (1) good biocompatibility and nontoxicity; (2) the scaffold shall have interpore connections with an aperture greater than 100 μm to provide effective oxygen and nutrients and to ensure good cell proliferation and migration; (3) it can be designed and shaped flexibly and it should be convenient to construct 3D scaffold quickly [7,11]. The progress of adipose reconstruction scaffold material depends on the progress of preparation technology. The biggest advantage of 3D scaffold is that it can provide a good biomimetic environment for cells and cytokines, etc. At present, the preclinical research on the combination of cells and biological scaffold material shows a good application prospect. The manufacturing technology of 3D adipose tissue engineering scaffold materials, the cells and growth factors used in adipose tissue reconstruction, and the application of 3D scaffold materials in adipose tissue reconstruction are introduced (Figure 1).

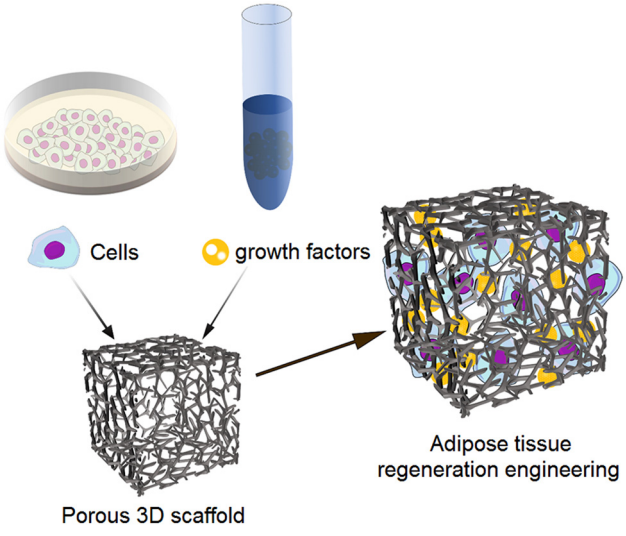


Figure 1: Cells, growth factors, and porous scaffolds constitute the adipose tissue regeneration engineering.

3.1 Preparation technology of 3D tissue engineering scaffold for adipose tissue reconstruction

At present, the methods applied to the construction of 3D biological scaffolds can be divided into two types: traditional construction and additive manufacturing forming technology (Figure 2). Traditional construction methods include solvent casting method and particle leaching method, freeze-drying method, and freeze-gel methods, which are simple and economical. For example, Phull et al. [44] used the solvent casting method and the particle leaching method to produce macroporous structures for soft tissue engineering. They used alginate microspheres as pore-foaming agents, and a mechanically stable scaffold material with large voids was prepared by gelatin for the surrounding matrix. The results showed that the gelatin matrix with ADSCs could support the growth and differentiation of cells along the fat line.

Additive manufacturing forming technology includes two kinds of technology methods of conventional 3D printing, which can fabricate post complex cells, growth factors, and direct 3D bio-fabrication. Additive manufacturing forming technology has the advantage that can make custom-made scaffold for patients according to their characteristics, and scaffold materials, seed cells, and growth factors can be prepared accurately; it is widely used in the manufacture of artificial organs, such as bone tissue, skin, trachea, and so on [45–47]. In conventional 3D printing manufacturing technologies, fused deposition modelling (FDM) technology based on extrusion molding can be used to fabricate 3D porous scaffolds of adipose tissue layer by layer. The main disadvantage of this method is that cells cannot be printed with materials and cells must be inoculated after printing. Chhaya et al. [48] added PLLA scaffold with an aperture more than 1 mm to human umbilical vein endothelial cells, and then subcutaneously implanted the scaffold into nude mice without thymus. Angiogenesis and adipose tissue formation were observed in all mammary scaffolds. Bio-fabrication is the application of “bio-ink” containing cell components to produce 3D scaffolds. Its advantage is that scaffolds and cells are prepared as “bio-ink” in advance, and then, they are simultaneously printed [49]. Current research on bio-fabrication focuses on how to solve the survival rate of cells and how do cells stay alive and differentiate into tissue after printing [50]. Gruene et al. [51] made 3D biological scaffolds by using laser-assisted methods in bio-fabrication, and the results showed that bio-fabrication had no effect on the differentiation potential and proliferation ability of stem cells.

3.2 Cells and cytokines of tissue engineering for adipose tissue reconstruction

3.2.1 ADSCs

Adipose tissue contains ADSCs. ADSCs are similar to bone marrow stem cells in their ability to differentiate and

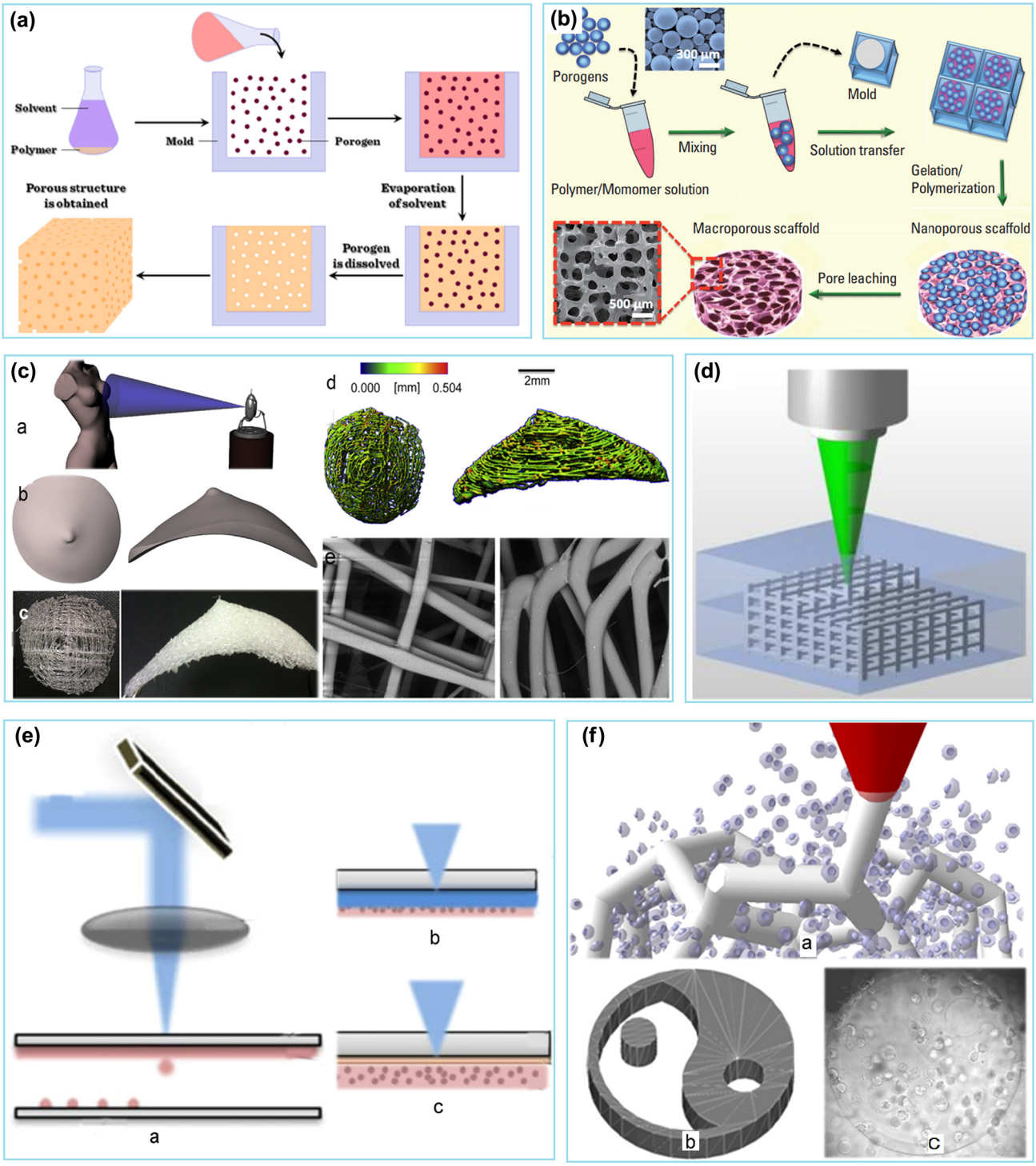


Figure 2: Figure 2: Examples of preparation methods of tissue engineering scaffolds. (a) Solvent casting [70]. (b) Particulate leaching method [71]. (c) Scaffold fabricated by FDM method: (a) laser scanning; (b) CAD model fabricating; (c) porous patient-specific scaffolds generating; (d) MicroCT scans show the filament and volume of the scaffolds; (e) SEM image bars and struts of the scaffold [46]. (d) Scaffold fabricated by laser fabrication approach [64]. (e) Scaffolds fabricated by the LAB method. (f) Two-photon polymerization (2PP) method: (a) schematic illustration showing 2PP structures (gray) fabricated by a focused laser beam (red) within the suspension of cells (blue); (b) CAD model used for laser cell encapsulation; (c) an optical online image of a 2PP-produced 400 μm wide Yin–Yang hydrogel structure with cells inside [73].

regenerate, and they can supplement lost volume at repair sites by proliferating and differentiating into adipocytes [52]. In addition, they also secrete various growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, fibroblast growth factor-2 (FGF-2), insulin-like growth factor 1, etc., playing an important role in adipose tissue regeneration and angiogenesis [53]. It has been reported that ADSCs mixed with HA filler can differentiate into fibroblasts, smooth muscle cells, preadipocytes, progenitor cells, endothelial cells, and stem cells after injection [54].

Several studies have shown that ADSCs promote the regeneration of adipose tissue. Yao et al. [55] encapsulated ADSCs of human in alginate microspheres and alginate/gelatin microspheres to simulate the natural fat lobule in adipose tissue. It was found that alginate/gelatin microspheres containing ADSCs showed higher cell proliferation and adipogenic differentiation than that of alginate microspheres. Turner et al. [56] prepared DAT as a substrate to induce ADSCs. The results showed that when ADSCs were inoculated on DAT microcarriers containing adipogenic medium, the adipogenic differentiation level was higher, while the gelatin microcarriers showed no substantial adipogenesis. In addition to promoting adipose tissue regeneration, ADSCs are related to promoting angiogenesis and collagen expression and have good biocompatibility and tolerance. Butler et al. [57] subcutaneously injected human microvascular endothelial cells (HMEC) and ADSCs coated with collagen hydrogel in mice, finding that combined transplantation with ADSCs had effects of anti-apoptosis and can promote angiogenesis on HMEC.

3.2.2 Growth factors of tissue engineering for adipose tissue reconstruction

Platelet-rich plasma (PRP) is a concentrated solution of a large number of platelets in a small volume of plasma. It has been reported that PRP contains a large number of cytokines that promote cell proliferation, differentiation, and angiogenesis. Liao et al. studied the effect of PRP on proliferation and adipogenic differentiation of ADSCs *in vitro* and found that PRP significantly increased the proliferation of ADSCs. Recently, Li et al. [58] studied the effects of PRP and conditioned medium on ADSCs in nude mice after subcutaneous injection of PRP combined with ADSCs. The results showed that the residual fat volume of PRP and ADSC group was significantly higher than that of other groups after 90 days. Therefore, the combination of PRP with ADSCs and fat transfer can significantly increase the proliferation and adipose differentiation of ADSCs and improve the survival rate of grafts after fat transplantation.

As a signaling protein, FGF has many functions such as development, metabolism, and adipokine. Adipokines are involved in the remodeling, lipogenesis, and angiogenesis of adipose tissue by autocrine/paracrine. Ogushi et al. [59] cross-linked ADSCs with FGF enzymes *in vivo* and *in vitro* and then loaded them into injectable carboxymethyl cellulose with phenolic hydroxyl for adipose tissue engineering. The results showed that the survival rate of adipocyte was 92.8%, and the adipocyte proliferation was good. In addition, after subcutaneous injection of Lewis rats for 10 weeks, new vascularized adipose tissue and lipogenesis were observed at the injection site. Therefore, FGF can be used as a component of ADSCs and fat transfer to promote adipose tissue generation and angiogenesis.

VEGF was first identified as an important role in angiogenesis in 1983 [60]. In addition, it has been found to play a role in wound healing, fatty tissue expansion, tumor growth, and age-related macular degeneration. Kim et al. [61] studied the effects of ADSCs and VEGF on myogenic differentiation in injectable heat-sensitive PEG–poly-e-caprolactone (PCL) hydrogels. The results showed that the combination of ADSCs and VEGF in the hydrogel showed obvious proliferation, differentiating into muscle tissue and vascularization enhancement after subcutaneous injection into the neck of mice for 4 weeks. Gorkun et al. [62] studied the effect of VEGF on endothelial cell differentiation after ADSCs or umbilical cord pluripotent MMSCs were cultured on unmodified and polyglycolated fibrin hydrogel. The results showed that poly(ethylene glycol) hydrogel had better multibranch formation than pure fibrin gels, and the differentiated ADSCs had stronger angiogenesis capacity than the differentiated umbilical MMSCs. Therefore, it can be believed that VEGF can play an important role in angiogenesis of ADSCs and fat transfer.

3.3 Application of 3D scaffolds for adipose tissue reconstruction

The application of 3D scaffold materials for adipose tissue reconstruction has made good progress, and some fruitful results of 3D porous scaffolds have been obtained *in vivo*. For example, (D,L)-lactide, GelMod, alginate, and DAT bioinks and PCL have been used in scaffold printing for adipose tissue engineering [7]. Chang et al. [63] used gelatin/HA cold gel scaffolds in nude mice, and the results showed that this type of scaffolds is expected to provide a stable structure and chemical environment, enabling cells to attach and proliferate, and supporting the biological functions and fat generation of ADSCs.

Ovsianikov et al. [64] prepared a methylacrylamide-modified gelatin (GelMod) scaffold with an aperture of 250 mm by bio-fabrication for adipose tissue engineering applications. The results showed that the crosslinking degree had no effect on the enzymatic degradation ability of the gel-based structure, and the scaffold successfully supported the adhesion, proliferation, and adipogenic differentiation of ASC.

However, the study of biological scaffolds still has many limitations. Compared with injectable materials, the placement of 3D biological scaffolds requires surgical incision, which is more complicated, and clinical verification is also required for the location and fixation of implants. The study of scaffold's microenvironments on regulation of cell fate is still a difficult issue [65]. Another difficulty that can be predicted is the fabrication of a tri-microbial scaffold, which uses materials that are same as injectable biomaterials. Its progress depends on advances in the fabrication of biomaterials. Therefore, it can be expected that the production time of biological scaffolds is longer and more difficult than that of *in situ* injectable materials [66]. The studies *in vivo* reported so far indicate that only very small amounts of material have been used in mouse or rat models. How these results will be applied to humans in future studies? And whether the hydrogels can achieve the same effect by increasing their volume? These problems are unclear. Hillel et al. [67] reported differences in inflammatory responses between mice and humans. Implants made of polyethylene glycol and HA caused a greater inflammatory response in human body Therefore, more clinical studies are needed to verify the effectiveness and safety of biological scaffold materials.

4 Summary and expectation

Injectable biomaterials are widely used in soft tissue reconstruction. Natural biomaterial has good biocompatibility, and it is a kind of natural degradable nonpermanent soft tissue filling material. However, natural biomaterials tend to have a higher absorption rate and are more difficult to produce long-lasting filling. Synthetic biomaterials have low biocompatibility, but some mechanical property, chemical property, and degradation property can be customized according to requirements. At present, many kinds of injectable scaffolds with good biological and mechanical properties have shown good effects in preclinical studies by combining relevant cells and cytokines with natural and synthetic biomaterials. ADSCs are pluripotent mesenchymal stem cells with the function of supplementing tissue defects through proliferation and maturation, so they are widely used in fat regeneration of various cell. The combination of ADSCs, growth factors, and biological scaffolds has shown a broad prospect in studies related to adipose tissue regeneration, but most of these studies are still in the preclinical stage. More clinical studies are needed to verify its safety and efficacy in the future.

Conflict of interest: The authors declare no conflict of interest regarding the publication of this paper.

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