Injectable Biomaterials in Plastic and Reconstructive Surgery: A Review of the Current Status

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Abstract

BACKGROUND: Injectable biomaterials have attracted increasing attention for volume restoration and tissue regeneration. The main aim of this review is to discuss the current status of the injectable biomaterials for correction of tissue defects in plastic and reconstructive surgery.

METHODS: Requirements of injectable biomaterials, mechanism of in situ gelation, characteristics, and the combinational usage of adipose-derived stem cells (ADSCs) and growth factors were reviewed.

RESULTS: The ideal injectable biomaterials should be biocompatible, non-toxic, easy to use, and cost-effective. Additionally, it should possess adequate mechanical properties and stability. In situ gelation method includes physical, chemical, enzymatic and photo-initiated methods. Natural and synthetic biomaterials carry their pros and cons due to their inherent properties. The combined use of ADSCs and growth factors provides enhanced potential for adipose tissue regeneration.

CONCLUSIONS: The usage of injectable biomaterials has been increasing for the tissue restoration and regeneration. The future of incorporating ADSCs and growth factors into the injectable biomaterials is promising.

Keywords Injectable biomaterials · Adipose-derived stem cells · Adipose tissue · Tissue regeneration · Plastic and reconstructive surgery

1 Introduction

Recently, injectable biomaterials have attracted increasing attention from physicians and researchers for correction of tissue defects from trauma, surgery, and intrinsic aging. In particular, tissue engineering techniques were developed to achieve the goal of volume restoration and tissue regeneration. As a noninvasive procedure, they have many advantages over invasive surgery. The concept of curing in situ, filling complex defects, and easy incorporation of desired cells or therapeutics makes injectable biomaterials undeniably favorable option over surgical procedures because the invasive surgical procedure increases recovery time and risk of complications.

The tissue defects are frequently accompanied by adipose tissue defects. The adipose tissue has several roles in

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energy consumption through lipid storage and metabolism as well as aesthetics, symmetry, and cushioning [1]. The development of adipocyte-based injectable biomaterials has an advantage since they can be formulated as combination system consisting of cells, growth factors and injectable biomaterials to fill the complex 3-dimensional shapes [2–4]. Adipose tissue contains adipose-derived adult stem cells (ADSCs) which possess the capability to differentiate into several mesodermal lineages, such as preadipocytes, chondrocytes, osteoblasts and myoblasts [5]. The ADSCs are relatively easy to harvest and carries a reduced risk of fibrous encapsulation than the subcutaneously implanted biomaterials [6].

In this review, injectable biomaterials that are clinically used in plastic and reconstructive surgery to restore and regenerate tissue defects are discussed. In particular, recent developments in various filler materials as injectable biomaterials including natural, synthetic, natural/synthetic hybrid and extracellular matrix (ECM)-derived biomaterials are detailed. Also, a combination of injectable biomaterials, cells and growth factors for adipose tissue regeneration are covered.

2 Ideal qualities of injectable biomaterials

There are several ideal qualities of injectable biomaterials: (1) they must possess biocompatibility without toxicity nor immunogenicity after degradation [7, 8]; (2) they must have adequate mechanical properties similar to the targeted tissue [4]; (3) their viscosity must be satisfactorily low to have a uniform dispersion before gelation of the injected biomaterials [9]; (4) they must have stability and tenacity to keep drugs and cells in the injected area; (5) they must have an acceptable amount of permeability, pore size and interconnectivity of pore in order to provide enough area for cellular activity, exchange of nutrients and oxygen; (6) they must be cost-effective with easy handling, and (7) they must be biodegradable to make space for cell growth and differentiation, and the newly formed tissues as illustrated in Fig. 1 [9]. The ideal qualities of injectable biomaterials are also briefed in Table 1.

3 Mechanism of *in situ* gelation of injectable biomaterials

There are several mechanisms of *in situ* gelation of injectable biomaterials by a physical method such as temperature, pH, electric field and magnetic field, chemical method, enzymatic method, photo method and hydrophobic interaction [10, 11]. In this section, we will briefly discuss the characteristics of each method.

3.1 Physical method

3.1.1 Temperature-induced gelation

Temperature-responsive injectable biomaterials such as chitosan [9], Poloxamer [9], poly(*N*-isopropylacrylamide) [9], poly(ethylene glycol) (PEG)–poly(L-lactic acid)–PEG triblock copolymer [9], polyphosphazene [9] and PEG-b-poly(D,L-alanine) [12] are widely used physiologically responsive hydrogels because they undergo gelation in response to temperature elevation, usually from low temperature to around 37 °C. Temperature proximate to the body temperature increases the hydrophobic interactions between the biomaterial chains [13]. Especially, injectable temperature-responsive hydrogels are very promising in adipose tissue engineering because they are safe to use in comparison with other stimuli such as ionic strength and pH. In injectable temperature-responsive hydrogels, the mixing of cells with biomaterials occurs at room temperature and a sol-gel transition at 37 °C [14].

3.1.2 pH-induced gelation

pH-responsive injectable biomaterials such as chitosan [9], *N*-palmitoyl-graft-chitosan [12] and poly(*N*-isopropylacrylamide-co-propylacrylic acid) [12] also have advantages of the physiological environment to get gelation at the physiological pH condition like temperature-responsive biomaterials [15]. The gelation occurred due to the increased hydrophobic interactions and hydrogen bonding between pH-responsive groups of biomaterials.

3.1.3 Photo-initiated gelation

Photo-initiated gelation occurs by the *in situ* formation of crosslinked hydrogels through photopolymerization of methacrylated glycol chitosan [15] or methacryloyloxy ethyl carboxyethyl chitosan [15] at physiological temperature and pH [16]. The period of light exposure can control the degree of polymerization and the mechanical properties of the hydrogels. Photoinitiators can show cytotoxicity due to the formation of free radicals [17].

3.1.4 Ionic-crosslinked gelation

Typically, there are two kinds of injectable biomaterial hydrogel by ionic interactions. One is alginate as a natural anionic polysaccharide that forms hydrogel upon interaction with divalent cations such as calcium and barium [18]. The other is chitosan as a natural cationic polysaccharide that can induce rapid gelation by mixing polyglutamate as a natural polyanionic polymer by the formation of polyelectrolyte complexes between cationic chitosan and

Fig. 1 Schematic illustration of injectable biomaterials. **A** The injectable biomaterials must have sufficiently low viscosity to be mixed with cells and growth factors to form a solution for *in vitro* or *in vivo* injection into the damaged tissues before gelation. **B** The injectable biomaterials must form hydrogels *in situ* to efficiently

encapsulate cells and growth factors upon *in vitro* physical stimuli or chemical reactions. **C** The injectable biomaterials must have a controllable degradation to make space for cell growth and differentiation and the newly formed tissues. Modified from Ref. [9]

Table 1 Requirements of injectable biomaterials

Non-toxic after degradation
Non-immunogenic
Adequate mechanical property
Mild gelation condition with stability and tenacity
Adequate porosity, pore size, and interconnectivity
Cost-effective with easy handling

anionic polyglutamate [19]. Also, rapid gelation of chitosan can be induced by mixing tri-polyphosphate [20, 21] or guanosine 5'-diphosphate [22] as an anionic crosslinker.

4 Chemical methods

4.1 Schiff base crosslinked gelation

Schiff base gelation of biomaterials was induced by chemical reaction between the amino groups (NH₂) of

gelatin and the aldehyde groups (CHO) in oxidized chondroitin sulfate [23], between NH₂ of chitosan and CHO of periodate oxidized heparin [24], between CHO of hyaluronic acid and hydrazide of hyaluronic acid-g-(hydrazide + dexamethasone) [25], and between the NH₂ of lysine of gelatin and the CHO in periodate oxidized alginate [26]. However, the biocompatibility of Schiff base crosslinked hydrogels should be checked for clinical application.

4.2 Michael addition crosslinked gelation

The Michael addition crosslinked gelation has been used for injectable hydrogel preparation because it has several advantages, such as relatively mild reaction with biomaterials, controlled reaction time and capability to formulate diverse types of bonds [9]. The gelation was induced by a reaction between thiolated chitosan and poly(ethylene glycol) (PEG) diacrylate [27], between thiolated hyaluronic acid and thiolated gelatin, and PEG-bis-maleimide [28], between thiolated heparin and PEG diacrylate [29], and

between thiolated gelatin and disulfide-containing PEG diacrylate [30].

4.3 Enzyme-catalyzed crosslinked gelation

Enzyme-catalyzed crosslinked gelation has been applied for tissue engineering because the gelation occurs at a mild condition. Horseradish peroxidase (HRP) is capable of gelation *in situ* in combination with phenol derivatives through the decomposition of hydrogen peroxide (H_2O_2) [31]. Hwang et al. [32] prepared *in situ* cross-linkable hydrogels by reaction of gelatin-PEG-tyramine and a mixture of HRP and H_2O_2 .

The human adipose-derived stem cells (h-ADSCs) and basic fibroblast growth factor (bFGF)-loaded hydrogel enhanced muscle redevelopment in a lacerated animal model. Jin et al. [33] prepared an injectable hydrogel by enzyme-catalyzed crosslinking of dextran-tyramine in the presence of HRP and H_2O_2 . The chondrocyte-seeded hydrogel enhanced the production of glycosaminoglycans (GAGs) and collagen type II than agarose gel. They also prepared injectable enzymatically crosslinked hydrogels by reaction of a mixture of dextran-tyramine and heparin-tyramine with a mixture of HRP and H_2O_2 . The chondrocyte-seeded dextran-tyramine/heparin-tyramine hydrogels induced more chondroitin and collagen than dextran-tyramine ones [34].

Lih et al. [35] prepared enzyme-triggered *in situ* cross-linked hydrogels based on chitosan-PEG-tyramine by crosslinking with a mixture of HRP and H_2O_2 . The hydrogels showed superior healing effects on rat skin than fibrin glue or cyanoacrylate. Ogushi et al. [36] developed an injectable *in situ* hydrogels by reaction of carboxymethylcellulose-tyramine with a mixture of HRP and H_2O_2 . The ADSCs-seeded hydrogel formed a new vascularized adipose tissue at injection site of Lewis rats. Xu et al. [37] prepared injectable hydrogels by reaction of hyaluronic acid-tyramine with a mixture of HRP and H_2O_2 . The interferon- α 2a-loaded hydrogels showed reduced cell density with higher number of apoptotic cells in tumor tissues of mice as well as a significant inhibition of angiogenesis in tumor tissues. The characteristics of *in situ* gelation of injectable biomaterials according to gelation mechanism are summarized in Table 2.

5 Injectable biomaterials

In this section, we will discuss the characteristics of injectable biomaterials as the clinically available soft tissue fillers for the tissue restoration.

5.1 Natural biomaterials

5.1.1 Collagen

More than 80% of the skin is composed of collagen and the main component of the connective tissues, ligaments, and tendons. The collagen among the natural extracellular matrices (ECMs) is one of the available biomaterials. Although the injectable collagen has rapid resorption rate, it was approved as the biologic dermal fillers by the Food and Drug Administration (FDA) [38–40].

There are three sources of collagen. One is bovine collagen, which is obtained from tendon, dermis, and bone of bovine [41]. The bovine collagens have been used as fillers since their approval by the FDA in 1981. Upon injection, they form a firm structure as fibrils with an axial periodicity of natural collagen to help rectify facial defects [42]. Currently, Zyderm® I and II are commercially available for the rectification of superficial lines as well as scars, crow's feet and periorbital lines [43]. The other is Zyplast®, which is used to cure deeper scar tissue as well as acne scars and vermilion border of lip [44]. However, they have shown adverse reactions such as foreign body reaction after the injection due to the xenogeneic properties of the material. The second one is porcine collagen, which is also obtained from tendon, dermis, and bone of swine. The porcine collagen has been recently promoted as an intradermal filler as the volume enhancer with the brand name of the Evolence™ [45]. TheraFill® showed higher Wrinkle Severity Rating Scale rating than bovine collagen filler without eliciting severe adverse reactions [46]. The third one is human collagen, which is obtained from human dermis. The collagen can also be obtained from the aspirate of adipose tissue after liposuction [47], or recombinant human collagen can be produced by yeast or bacteria [48]. Currently, Cosmoderm® and Cosmoplast® are commercially available collagen products of human origin after receiving FDA approval. The Cosmoplast® is cross-linked between lysin residues on collagen by glutaraldehyde to slow down the degradation. The Cosmoderm® is used for the treatment of superficial wrinkles whereas the Cosmoplast® is used for the cure of deep scars and deep wrinkles. They are both more expensive than bovine collagen and porcine collagen.

5.2 Hyaluronic acid (HA)

The HA is also one of ECMs found in the many body parts as a naturally occurring glycosaminoglycan usually obtained from either bacteria or rooster combs. It shows an superb immune profile with negligible allergy reactions [49]. Currently, Eleven HA fillers are approved by the FDA as soft tissue fillers [50]. Among approved HA fillers, Restylane® and Juvéderm™ are very commonly used as

Table 2 Characteristics of *in situ* gelation of injectable biomaterials according to gelation mechanism

Gelation	Method	Advantages	Disadvantages	Ref.
Physical	Temperature	Mixing of cells with biomaterials at room temperature	Reversible sol-gel transition	[13]
	pH	Gelation at the physiological environment	Low cell viability at acidic condition	[14]
	Photo	<i>In situ</i> formation of crosslinked hydrogels	Cytotoxicity by photo initiators and photo source	[15]
Chemical	Ionic	Rapid gelation	Rapid release of growth factors	[17]
	Schiff base	The chemical reaction between amino groups and aldehyde groups	Biocompatibility of used chemicals should be checked	[22]
	Michael addition	Mild chemical reaction	Biocompatibility of used chemicals should be checked	[25]
Enzyme	Enzyme	The occurrence of gelation at the mild condition	Used chemicals should be approved for clinical application	[29]

volume enhancer and soft tissue contouring. They are classified as temporary fillers because they do induce permanent tissue restoration by a single injection. Therefore, many types of research are focused on the reducing of the degradation rate of HA to ensure volume persistence along with the improvement of the biological performance [14]. Two strategies have been tried to slow down the degradation rate of the HA. One is a dual crosslinking method [51, 52]. The other is a combination of HA with gelatin [53], chitosan [54], cellulose [55] and PEG [56]. The combination of HA with another biomaterial have shown excellent biocompatibility as well as retainment of significantly larger average volumes compared with HA-based fillers [57]. Nonallergic local side effects include pain, bruising and transient edema at the sites of HA [58]. Another FDA-approved HA, Hylaform® or Hylaform Plus® showed similar efficacy for nasolabial folds compared to Zyplast® with fewer adverse effects and no allergic reactions [59]. Perlane® marketed by the same manufacturer of the Restylane® is composed of biphasic gels comprising of particles suspended in a solution which is cross-linked with 1,4-butanediol diglycidyl ether to lessening the rate of degradation and to increase the surface area of the molecule [60]. The Perlane® has been used as deep dermis, and superficial subcutis whereas Restylane® has been used for mid-to-deep dermal soft tissue fillers due to the smaller particle size ranging around 400 µm [59]. Another soft filler of Belotero® approved by FDA in 2011 has been clinically used for very fine lines such as the forehead, vermillion border and tear trough as it has a higher concentration of non-cross-linked HA [60].

5.3 Autologous fat grafting

The autologous fat grafting has been performed since 1893. Autologous fat is biocompatible, inexpensive, easy to harvest, and nonimmunogenic. The longstanding endurance of transplanted fat is still variable and

unpredictable [61]. Also, the fat grafting can be used as a reconstructive, and cosmetic technique for patients with contour deformities and volume loss by tumor expiration, trauma, and natural aging [62]. In plastic surgery, lipofilling has been widely used in breast reconstruction [63], facial hemiatrophy, and lipodystrophy [64], rejuvenation of the aging face [65] and hands [66] in the treatment of depressed scars [67]. It has also been used in the treatment of scleroderma because by the formation hypodermis, dermis, and epidermis due to the regenerative capacity of the ADSCs [68]. However, several factors such as atraumatic harvest of fat, minimal manipulation of harvested fat, a small aliquot of injection close to muscles for enhancing revascularization, and careful layering of the fat for achieving the desired effect should be considered for the higher survivability of the injected fat [69]. Interestingly, the fat grafting prepared by the Puregraft™ system showed considerably lower blood cell and free lipid content with enhanced adipose tissue viability [70]. Also, REVOLVE™ system showed significantly less blood cell debris, greater content of adipose tissue and a lower quantity of free oil [71].

5.4 Synthetic biomaterials

5.4.1 Poly(methyl methacrylate) (PMMA)

PMMA is a man-made, non-biodegradable and biocompatible polymer used in numerous medicinal devices. It has been used as intradermal filler. The PMMA microspheres trade named as Arteplast® have been found to last over 6 months [72]. It caused foreign body reactions owing to the PMMA microspheres being less than 20 µm which elicited immune reactions such as phagocytosis and inflammation [73], showing that the size of PMMA microspheres was critical to avoid phagocytosis by macrophages. Artefill® as a third generation PMMA microspheres with sizes of 30–50 µm and collagen filler has been

used as volume enhancer and contouring of nasolabial, radial upper lip lines, glabella lines, and corner of mouth lines. It has been reported to have less risk of phagocytosis and decreased granuloma formation [74]. Artcoll[®] as a second generation PMMA microspheres and collagen filler demonstrated a greater enhancement in nasolabial folds compared to collagen filler itself with less adverse effects [75].

5.4.2 Poly(L-lactic acid) (PLA)

The PLL originally synthesized from α -hydroxy acid as a biodegradable thermoplastic polymer has been widely used as suture materials, resorbable plates, and screws in orthopedic, neurologic, and craniofacial surgery [76]. Sculptra[®] was approved in 2008 by the FDA for soft tissue restoration of lipoatrophy in HIV patients. Its usage was expanded for filling the deep dermal or subcutaneous layer and for restoring facial fat atrophy [77]. Once injected, the PLA generated instant volumization secondary to the properties associated with the PLA microparticles [78]. The effect of volumization disappeared after few months due to degradation of the microparticles and resulted in dermal fibroplasia [79]. Also, the Sculptra[®] functions as biostimulatory agent by stimulating the creation and vascularization of collagen [74]. Therefore, the Sculptra[®] was also approved as a cure for nasolabial folds, facial wrinkles, lines and enhancement of contour deficiencies [80].

5.4.3 Calcium hydroxylapatite (CH)

The CH and its byproducts naturally located in human bone and dental enamel are very biocompatible because they have the structural similarity to the mineral part of bone and comprises of calcium and phosphate ions [81]. The Radiesse[®] consisted of CH microspheres suspended in carboxymethyl cellulose gel. It was approved in 2006 and has been used for the rectification of wrinkles and volumization of facial soft-tissue [82]. The CH microspheres in the gel endorse the formation of new tissue by collagen deposition. The gel degrades with in 2–3 months and the byproducts (calcium and phosphate ions) would be eliminated from the body through phagocytosis by macrophages [83]. Also, the Radiesse[®] was superior to the treatment of nasolabial folds compared to collagen [84].

The characteristics of injectable biomaterials as clinically available soft tissue fillers are summarized in Table 3. Also, injectable soft tissue fillers by dates of FDA approval are listed in Table 4 [80].

6 Combination of cells and growth factors with injectable ECMs

The combination of cells and growth factors with injectable ECMs is very powerful for providing adipose tissue regeneration because the regenerative characteristics of ADSCs, ease and elevated accessibility of adipose tissue and great release of growth factors from ECMs, which will allow us to achieve high retention rate and longevity in adipose tissue regeneration [85–87]. This section covers effects of cells and growth factors with ECMs on the adipose tissue regeneration.

6.1 Combination of cells

There are multiple cell sources such as a terminally differentiated adipocyte, preadipocytes of rat, mouse, and human, which are committed to the adipocyte cell lineage, adipose-derived stromal cells (ASCs) and ADSCs for adipose tissue engineering [88]. This section will restrict cell sources to the ADSCs as well as ASCs due to page limitation.

6.2 ADSCs

The ADSCs have been widely used for adipose regeneration among several sources because they are multipotent mesenchymal stem cells (MSC), and they are more easily accessible, isolable and have considerably lower morbidity. They exhibit features similar to bone marrow stem cells (BMSCs) [89]. Also, they are prone to replenish lost volume through multiplying and maturation into adipocytes [90]. Furthermore, they secrete several growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor-2 (FGF-2), and insulin-like growth factor 1 (IGF-1), which plays an vital role in adipose tissue regeneration and angiogenesis [91].

ADSCs mixed with HA filler generated various tissues after injection. They can differentiate into a heterogeneous cell population such as fibroblast, smooth muscle cells, preadipocytes, progenitor cells, endothelial cell, and stem cells [92].

Butler et al. [93] subcutaneously injected ASCs in small collagen hydrogel rods coated with adipose-derived human microvascular endothelial cells (HMEC) into SCID/Bg mouse. The results indicated that HMEC on modules containing harvested ASCs detached from modules and formed vessels as early as 3 days, and the vessels stayed for up to 90 days whereas the HMEC on modules without harvested ASCs continued to attach to the module surface

Table 3 Characteristics of injectable biomaterials as clinically available soft tissue fillers

Source	Material	Trade name	Uses	Pros	Cons	Ref.
Natural (Biologic)	Collagen	Zyderm® I and II	Soft-tissue augmentation (formation of rigid structure as fibrils)	Correction of facial soft tissue defects	Immunogenic reactions	[39]
		Zyplast®	Soft-tissue augmentation (deep scar tissue)	Correction of facial soft tissue defects	Immunogenic reactions	[40]
		Evolence™	Volume enhancer	Less immunogenic than bovine	Mild immunogenic reactions	[41]
		TheraFill®	Filling of wrinkle	Less immunogenic than bovine	Mild immunogenic reactions	[42]
		Cosmoderm®	No skin test required	No skin test required	Mild immunogenic reactions	[44]
	HA	Restylane® and Juvederm™	Volume enhancer, usable in superficial and deep tissue (along bone)	No skin test required Minimal allergic reactions	Mild swelling	[13, 47]
		Hylaform®		Long-lasting		
Synthetic	PMMA	Arteplast®	Volume and contouring	Easy fabrication	Inconsistent quality Donor site morbidity (bruising, contour deformity)	[83]
		ArteColl®	Deep folds (Nasolabial) and acne scars	Long-lasting	Foreign body reaction—risk of granuloma	[71]
	PLA	Sculptra®	Restoration of facial fat loss, usable in deep tissue (along bone)	No skin test	Foreign body reaction—risk of granuloma Bumps Requires skin test	[74]
					Rapid degradation Irregularities	[76]
	CH	Radiesse®	Correction of moderate-to-severe wrinkles, usable in deep tissue (along bone)	Long-lasting	Inflammatory reactions More swelling Irregularities	[81]

ADSC adipose-derived stem cells, CH calcium hydroxylapatite, HA hyaluronic acid, PLA poly(L-lactic acid), PMMA poly(methyl methacrylate)

at day 3, demonstrating co-transplantation with ASCs had an anti-apoptotic and proangiogenic effect on the HMEC.

Lee et al. [94] subcutaneously implanted HA filler/ASCs into the backs of 4 nude mice. The results showed that collagen fibers and fibroblasts were originated around newly formed vascular structures after 16 weeks by the HA/ASCs whereas only HA filler did not show cells or HA particles, demonstrating generation of fibrovascular tissue by HA/ASCs.

Yao et al. [95] encapsulated human ADSCs (hADSCs) in alginate and alginate/gelatin microspheres to mimic a natural fat lobule in adipose tissue. The hADSCs-loaded alginate/gelatin microspheres showed much higher cell proliferation and adipogenic differentiation than alginate ones due to even distribution of adipocytes although they did not check *in vivo*. Similarly, Kim et al. [96] encapsulated cryopreserved hADSCs into injectable and degradable oxidized alginate hydrogels prepared susceptible to

hydrolysis by periodate oxidation of alginate for engineering adipose tissue. The results showed that newly generated adipose tissues were soft and semitransparent in mice, grossly resembling adipose tissue at 10 weeks after injection of cell-loaded gels subcutaneously into the chest wall of nude mice.

Turner et al. [97] fabricated human decellularized adipose tissue (DAT) as an inductive substrate for hADSCs. The results indicated that hADSCs seeded on the DAT microcarrier with adipogenic differentiation medium showed high levels of adipogenic differentiation in a low-shear spinner culture system whereas gelatin microcarrier did not show substantial adipogenesis. Also, the pre-seeding of DAT microcarriers with allogenic rat ADSCs in a subcutaneous Wistar rat model improved angiogenesis within the implant region without immunogenicity or cytotoxicity.

Table 4 Lists of injectable soft tissue fillers by dates of FDA approval (modified by Ref. [80])

Source of product	Trade name of product (manufacturer)	Approval year
Bovine collagen	Zyderm 1 (Inamed/Allergan)	1981
Bovine collagen	Zyderm 2 (Inamed/Allergan)	1983
Bovine collagen	Zyplast (Inamed/Allergan)	1985
Human collagen	Cosmoderm (Inamed/Allergan)	2003
Human collagen	Cosmoplast (Inamed/Allergan)	2003
Hyaluronic acid	Restylane (Medicis Aesthetics) ^a	2003
Animal derived hyaluronic acid	Hylaform (Inamed Corp)	2004
Non-animal derived hyaluronic acid	Captique (Genzyme Corp)	2004
Poly(L-lactic acid)	Sculptra (Valent Pharmaceuticals) ^a	2004
Human collagen	Cosmoderm 2 (Inamed/Allergan)	2005
Non-animal hyaluronic acid	Juvederm ultra (Allergan) ^a	2006
Non-animal Hyaluronic Acid	Juvederm ultra plus (Allergan) ^a	2006
Poly(methyl methacrylate)	Artefill (Suneva Medical) ^a	2006
Calcium hydroxylapatite	Radiesse (Merz Aesthetics) ^a	2006
Non-animal hyaluronic acid	Perlame (Medicis Aesthetics) ^a	2007
Non-animal hyaluronic acid	Eleveess (Anika Therapeutics)	2007
Non-animal HYALURONIC ACid	Prevelle silk (Mentor corp)	2008
Porcine collagen	Evolence (Col Bar Lifescience)	2008
Non-animal hyaluronic acid	Hydrelle (Anika Therapeutics)	2009
Poly(L-lactic acid)	Sculptra Aesthetic (Vacant Pharmaceuticals) ^a	2009
Non-animal hyaluronic acid with lidocaine	Juvederm XC (Allergan) ^a	2010
Non-animal hyaluronic acid with lidocaine	Restylane-L (Medical Aesthetics) ^a	2010
Non-animal hyaluronic acid with lidocaine	Perlame-L (Medical Aesthetics) ^a	2010
Non-animal hyaluronic acid	Belotero (Merg Pharmaceuticals) ^a	2011
Autologous fibroblasts	LaViv (Fabrocell Technologies) ^a	2011
Non-animal hyaluronic acid	Juvederm Voluma (Allergan)	Pending
Non-animal hyaluronic acid with lidocaine	Juvederm Voluma-XD (Allergan)	Pending

^aProducts are currently available

Xu et al. [98], reconstructed epidural fat using ADSCs-seeded porous PLGA scaffold after implantation of them in a rabbit dorsal laminectomy model. It was found that the flaw fixed with engineered adipose created a continuous linear adipose tissue along the spinal cord at 24 weeks whereas scar tissue of coarse and high density was produced in laminectomy site in PLGA itself.

Lee et al. [99] compared regeneration of volume-stable adipose tissues between polycaprolactone (PCL)/ASCs and fibrin gel/ASCs after subcutaneous transplantation into the dorsal pockets of athymic mice. The results showed that adipose tissue areas of PCL/ASCs were significantly larger than those of fibrin/ASCs at 6 weeks after implantation, showing important parameter of scaffold composition on tissue growth.

Wang et al. [100] compared adipogenic differentiation ability of ADSCs between adipose tissue extract microparticle scaffolds originated from human adipose tissue and small intestine submucosa microparticle from

porcine jejunum as the ECMs after implantation of cell-loaded scaffolds into nude mice subcutaneously. The results indicated that the cells proliferated quicker on the adipose tissue scaffolds than on small intestine submucosa ones and formation of adipocytes at the adipose tissue scaffolds was closer to that of normal human fat tissue compared with that of small intestine submucosa ones.

Yeon et al. [101] synthesized poly(ethylene glycol)-poly(L-alanine) (PEG-PA) diblock copolymer injectable thermogel to study differentiation ability of ADSCs after loading cells in the gel. The results indicated that the cells exhibited high expression of type II collagen and expressed myogenic differentiation factor I and type III β -tubulin *in vitro*. Also, the cells largely exhibited chondrogenic biomarkers in BALB/c mice after injection of ADSC-loaded PEG-PA gel subcutaneously.

Cheung et al. [102] prepared injectable composite hydrogel scaffolds incorporating DAT and ADSCs using photo-cross-linkable methacrylate glycol chitosan (MGC) or methacrylate chondroitin sulfate (MCS) for soft tissue

engineering. The results indicated that the incorporated DAT into the scaffolds enhanced cell viability, adipogenesis, and retention *in vitro* although the MCS composites with 5 wt% DAT showed higher cell viability and adipogenic differentiation. Also, the MCS composites showed higher implant integration and adipogenesis in a subcutaneous Wistar rat, demonstrating the possibility of soft tissue regeneration for plastic and reconstructive surgery.

Lequeux et al. [103] intradermally injected Cytocare® as an injectable medical filler composed of HA, amino acids, vitamins and mineral salts with ASCs into the nude mice. The results showed that the injection of ASCs with Cytocare® generated more density of adipocytes with good biocompatibility and excellent tolerance than Cytocare® itself.

Jaikumar et al. [104] prepared injectable composite hydrogels incorporating fibrin nanoparticles using ionic cross-linkable alginate/*O*-carboxymethyl chitosan (*O*-CMC) or alginate/poly(vinyl alcohol) (PVA) for adipose-tissue engineering. The scaffolds showed good survival, proliferation, and differentiation into adipocytes *in vitro* although fibrin-loaded hydrogel scaffolds showed better differentiation ability, demonstrating the potential for soft tissue regeneration.

Domingues et al. [55] prepared injectable composite hydrogels composed of adipic acid dihydrazide-modified hyaluronic acid (ADH-HA) and aldehyde-modified cellulose nanocrystals (A-CNCs) to reinforce composite hydrogels in soft tissue engineering. The ADSC-loaded composite hydrogels showed the ability to spread the cells within the gels and enhanced proliferative activity *in vitro* owing to superior structural integrity and interaction of microenvironmental cues with CNC's sulfate groups although they did not perform *in vivo* study.

Lu et al. [105] injected ECM powders from porcine adipose tissue with ASCs into the dorsum of mice. The

results showed that the presence of the ASCs loaded in the ECM powders promoted more adipogenesis, angiopoiesis and adipogenic genes than ECM powders, demonstrating an efficiency.

Recently, Zhang et al. [106] seeded ADSCs into poly(L-glutamic acid) hydrogel porous scaffold to construct vascularized adipose units by inducing ADSC spheroids because the vascularization is very important for regeneration of adipose tissue. The results showed that the scaffolds made ADSC aggregate to form spheroids due to the swollen hydrophilic network property of the scaffolds. Also, the significant angiogenesis was found in the adipogenic induced group after 12 weeks post-implantation of the scaffold in the dorsum of nude mice.

6.3 Combination of cells and growth factors

The ADSCs are a particularly attractive cell source for adipose tissue engineering due to their easy isolation, relative abundance, and secretion of growth factors. However, the potential of ADSCs is dependent on the donor's medical condition because diabetic donors showed reduced proliferative potential and a small proportion of stem cell marker-positive cells [107]. Therefore, incorporation of growth factors with scaffolds and cells is desirable to regulate the maintenance of ADSC stemness. In this section, the incorporation of combined growth factors and ADSCs into the scaffolds for the adipose tissue regeneration will be discussed.

6.4 Platelet-rich plasma (PRP)

It is already reported that the PRP as a concentration of platelets in a small volume of plasma has six major growth factors such as platelet-derived growth factor (PDGF),

Fig. 2 **A** Comparison of average retained fat volumes in experimental and control groups after 10, 30, 60 and 90 days. **B** Changes in capillary number in the experimental and control groups at the end of

10, 30, 60 and 90 days. Reproduced with permission [113] of John Wiley and Sons, Inc.

Fig. 3 Effect of gel containing F/P MPs and FGF-2 *in vivo* on granulation thickness. Black, dark gray, light gray, and white bars show gel containing both F/P MPs and FGF-2, F/P MPs, FGF-2, and none, respectively. Reproduced with permission [117] of John Wiley and Sons, Inc.

basic fibroblast growth factor (bFGF), VEGF, epidermal growth factor (EGF), IGF-1 and transforming growth factor- β (TGF- β) [103]. Also, it stimulates cell proliferation, differentiation and neoangiogenesis [103].

Cervelli et al. [108] reported that addition of insulin ameliorated patients' 1-year PRP-induced fat graft volume maintenance and potentiated adipogenesis in human ADSCs via an FGF-1 and epidermal growth factor receptor-2 (ErbB-2)-regulated Akt mechanism. Also, they reported that the combined use of stromal vascular fraction (SVF) and PRP with fat grafting showed a 69% protection of the contour and of three-dimensional volume upto 1 year compared with those treated with a fat graft, who showed a 39% maintenance [109]. Furthermore, they reported that the combined use of adipose-derived SVF and PRP with fat graft improved more regeneration of scars on patient's face after 1 year compared with the control group treated with fat graft [110].

Liao et al. [111] investigated the effect of PRP on proliferation and adipogenic differentiation of ADSCs *in vitro* because PRP enhanced fat graft survival after lipotransfer. It was found that the PRP significantly increased proliferation of ADSCs even in the presence of antiproliferative, proadipogenic media whereas it inhibited adipogenic differentiation in adipogenic media through down-regulation of bone morphogenetic protein receptor IA (BMPR IA) and fibroblast growth factor receptor 1 (FGFR 1). Similarly, Seyhan et al. [112] studied the effect of combined use of PRP and ADSCs on fat graft survival to increase the viability of fat grafts. The results showed that the mean weight and volume of the fat grafts were the highest in the combined group compared with another single group after injection of combined PRP and ADSCs into the rat scalp.

Recently, Li et al. [113] studied the effect of PRP and conditioned medium on the characteristics of ADSCs after subcutaneous injection of combined PRP and ADSCs into nude mice. The results showed that the residual fat volume of $10^5/\text{ml}$ PRP + ADSCs group was significantly higher than that of other groups after 90 days. Also, normal adipocyte area and capillary formation were significantly increased in the combined group at a cell density of $10^5/\text{ml}$ compared with other groups as shown in Fig. 2.

6.5 FGF

The FGF as one of signaling proteins has various functions such as development, metabolism, and adipokine [114]. The adipokine plays roles in remodeling, adipogenesis, and angiogenesis of adipose tissue via an autocrine/paracrine manner [115].

Ogushi et al. [116] loaded ADSCs into injectable carboxymethyl cellulose having phenolic hydroxyl groups after enzyme-crosslinking by incorporation of FGF *in vitro*

and *in vivo* for adipose tissue engineering. The results indicated that 92.8% cell viability, good proliferation, and adipogenic differentiation were obtained *in vitro*. Moreover, visible new vascularized adipose tissue and adipogenesis were formed at the injection site at 10 weeks after the subcutaneous injection into Lewis rats.

Sumi et al. [117] loaded ADSCs into plasma gel with fragmin/protamine microparticles (F/P MPs) and FGF-2 in inbred male Fisher rats. The results indicated that the ADSCs grew faster under 3D-culture conditions in low plasma gel with F/P MPs and FGF-2 *in vitro*. Moreover, the number of capillaries, as well as the thickness of tissue

Fig. 4 **A** Effect of the bFGF dose on the weight of adipose tissue newly formed 6 weeks after implantation of CGS, incorporating ASCs impregnated with NSS (A), 0.1 $\mu\text{g}/\text{cm}^2$ bFGF (B), 1 $\mu\text{g}/\text{cm}^2$ bFGF (C), 7 $\mu\text{g}/\text{cm}^2$ bFGF (D), 14 $\mu\text{g}/\text{cm}^2$ bFGF (E), a collagen sponge incorporating ASCs and 1 $\mu\text{g}/\text{cm}^2$ free bFGF (F) and a collagen sponge incorporating ASCs and NSS (G). **B** The area of adipose tissue newly formed 6 weeks after implantation of CGS incorporating ASCs impregnated with NSS (A), 0.1 mg/cm^2 bFGF (B), 1 mg/cm^2 bFGF (C), 7 mg/cm^2 bFGF (D), 14 mg/cm^2 bFGF (E), a collagen sponge incorporating ASCs and 1 mg/cm^2 free bFGF (F) and a collagen sponge incorporating ASCs and NSS (G). Reproduced with permission [118] of John Wiley and Sons, Inc

granulation, was significantly higher in plasma gel with F/P MPs and FGF-2 than those in plasma gel either FGF-2 or F/P MPs at 8 days after injection of them into the back subcutis of rats 5 cm above the tail root as shown in Fig. 3.

Ito et al. [118] loaded ADSCs into collagen/gelatin sponge with FGF to get adipogenesis in nude mice after implantation into the back subcutis of nude mice. The results indicated that the weight and area of regenerated adipose tissue were the largest in the combined use of ADSCs and $1 \mu\text{g}/\text{cm}^2$ FGF group at 6 weeks after implantation as shown in Fig. 4.

6.6 VEGF

The VEGF was first identified as a role player in vasculogenesis and angiogenesis in 1983 [119]. Also, it was found to have a role in wound healing, adipose tissue expansion, tumor growth and age-related macular degeneration [120].

Kim et al. [121] studied the effect of ADSCs and VEGF on the myogenic differentiation in the

injectable thermosensitive PEG-PCL hydrogel. The results indicated that the combination of ADSCs and VEGF into the hydrogel showed significant proliferation, differentiation into muscle tissue and enhanced vascularization at 4 weeks after subcutaneous injection into the necks of mice, the showing of the possibility of adequate blood supply to sustain cell viability during tissue growth as shown in Fig. 5.

Colazzo et al. [122] studied the effect of combined use of shear stress and VEGF as mechanical and chemical stimulation on endothelial differentiation of ADSCs after seeding the cells onto collagen or acellular aortic valve matrices for up to 14 days. The results indicated that the ADSCs subjected to the shear stress and VEGF expressed specific endothelial markers and maintained the phenotype when seeded onto the scaffolds, demonstrating getting an efficient source of endothelial-like cells for tissue engineering based on ADSC in the presence of shear stress and VEGF although they did not check *in vivo*.

Recently, Gorkun et al. [123] studied the effect of VEGF on endothelial cell differentiation after culturing of ADSCs

Fig. 5 SMA- α (Cy3) expression and nuclei (DAPI) were detected by immunofluorescence staining. **A–C** MPEG-PCL alone, **D–F** ADSC/MPEG-PCL without VEGF and **G–I** ADSC/MPEG-PCL with

VEGF165 after 2, 4 and 6 weeks. VEGF165 treatment enhanced blood vessel formation. Arrow indicates blood vessel. Reproduced with permission [121] Copyright © 2018, Elsevier

or umbilical cord (UC) multipotent mesenchymal stromal cells (MMSCs) onto non-modified and PEGylated fibrin hydrogels. The results indicated that the PEGylated hydrogel showed better the formation of multibranched cords than the pure fibrin gel and the differentiated ADSCs had higher angiogenic ability than the differentiated UC MMSCs.

7 Conclusions and future perspectives

A wide range of injectable and biocompatible biomaterials such as natural or synthetic biomaterials have been probed for application in tissue regeneration. The natural biomaterials, being naturally biodegradable, have been actively used as non-permanent soft tissue filler materials due to biocompatibility and biological properties. They lack the structural integrity to create the lasting effect whereas synthetic biomaterials can be specifically tailored to possess certain mechanical properties, chemical properties and degradability of the materials although they do not have cell adhesiveness.

The promising results have been reported by combined utilization of natural and synthetic biomaterials to successfully generate injectable scaffolds with both advantageous biological properties and mechanical properties. Within the approach of injectable biomaterials, research is presently changing toward more progressive techniques that have physically, chemically and enzyme-catalytically crosslinked hydrogel systems. Ideally, these approaches should integrate well with the host tissue to provide the mechanical support and to generate functional tissue in place of damaged tissues. The ADSCs have been widely applied for adipose regeneration among several cell sources because they are multipotent MSC and naturally inclined to replenish lost volume through proliferation and maturation. Notably, the combination of ADSCs and growth factors in the injectable ECMs is very promising in adipose tissue regeneration since the ADSCs themselves can secrete several growth factors.

One important challenge, advanced 3D bioprinting techniques will be ultimately applied to injectable biomaterials. The printed biomaterials in the presence of cells to increase retention rate of the tissue regeneration will be available with high clinical feasibility. Another critical challenge, incorporation of various advanced imaging techniques including magnetic resonance imaging will be utilized to investigate *in vivo* characteristics of the biomaterials.

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Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest.

Ethical statement There are no animal experiments carried out for this article.

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