Adipose regeneration and implications for breast reconstruction: update and the future

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Abstract: The evolution of breast reconstruction and management of breast cancer has evolved significantly since the earliest descriptions in the Edwin Smith Papyrus (3,000 BC). The development of surgical and scientific expertise has changed the way that women are managed, and plastic surgeons are now able to offer a wide range of reconstructive options to suit individual needs. Beyond the gold standard autologous flap based reconstructions, regenerative therapies promise the elimination of donor site morbidity whilst providing equivalent aesthetic and functional outcomes. Future research aims to address questions regarding ideal cell source, optimisation of scaffold composition and interaction of de novo adipose tissue in the microenvironment of breast cancer.

Keywords: Adipose derived stem cell (ADSC); adipose; breast reconstruction; regenerative medicine

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Background: breast cancer and the need for reconstruction

As the second most common cancer worldwide (11.9%), each year 1.7 million women receive a new diagnosis of breast cancer (1,2). While advances in treatment have meant an overall reduction in mortality [522,000 deaths per year (1)], the global burden of this disease continues to be significant (3). In Britain, the age standardised incidence and mortality remains one of the highest in the world with around 14,000 women losing their lives every year to this disease (4). For those that survive, the morbidity is life changing, with physical, emotional and psychological aspects requiring multidisciplinary management (3,5-8). The treatment of breast cancer has evolved significantly; our understanding of the pathophysiology and molecular aetiology has advanced the way in which primary and adjuvant therapy is designed and delivered (9,10). Personalised treatment is playing an essential role in improving life expectancy, which has doubled in the last 50 years (9).

In the UK, of the approximately 46,000 women diagnosed annually with breast cancer, around 40% undergo mastectomy as their primary therapeutic procedure (11,12) and 30% of these have either immediate or delayed reconstruction. NICE guidance recommends that all women undergoing breast cancer surgery should be offered immediate reconstruction at their initial operation (12,13). The caveat being that where patients are primarily undergoing adjuvant treatment, this can be delayed to such time that reconstruction becomes an option. The guidance goes on to recommend that even if reconstruction is not available locally, patients should be provided with all the
options in order to make an informed choice (13). It may be surprising then, that of all women eligible; only 21% undergo immediate reconstruction (11). Patients who opt for reconstruction (either immediate or delayed) have been shown to have higher levels of well-being (emotionally and sexually) when compared to those undergoing mastectomy alone (12). It is important, therefore, as reconstructive techniques advance, to not only offer patients a choice of reconstructive options, but involve them in the decision making surrounding their treatment (Figure 1).

The earliest documented cases of breast cancer is The Edwin Smith papyrus (3,000 BC) which details eight patients with tumours of the breast that were clearly distinct from the surrounding tissue (14). Seen for many centuries as an incurable disease, early surgical intervention was often difficult and postponed for fear of shortening the life of the patient (14); Galen [120-200 AD] was one of the first to promote clear margins, identifying the ‘crab-like’ projections of the invading tumour (2,14). Work done by French surgeons Pare [1510-1590] and Cabrol [1549-1610] greatly informed surgical techniques by recognising the involvement of axillary nodes in the spread of disease (15). The advancements in anatomical understanding and development of mastectomy instruments in the 1500-1700s were limited until the invention and popularisation of anaesthesia by Morton in 1846 and asepsis by Lister in 1867 (2,9,14,16).

The radical mastectomy developed by Halstead in the 1880s became the gold standard in breast cancer surgery for the next seven decades (9,14,17). Despite 75% of his patients having axillary node disease at the time of operation, Halstead was able to demonstrate a 40% cure rate at 5 years (14). The fear of leaving disease behind with less aggressive approaches meant that it wasn’t until the 1970s that alternatives to radical surgery were considered. Trials in Europe and the US were beginning to demonstrate comparable outcomes to Halstead could be achieved with breast conserving surgery (BCS) and radiotherapy (18-20).

The shifting attitude meant that reconstruction, which had been previously thought of as a ‘luxury operation’ became a more integrated part of the surgical management of these patients (2,21). Czerny is credited as the first to demonstrate mound reconstruction in a patient with benign disease by re-purposing a lipoma from their flank (22). The work done by Tasini and Ombredanne in the early 1900s demonstrated new techniques using local myocutaneous flaps to recreate the breast (23,24). This evolution continued throughout the 1900s; with the previously described tubed abdominal flap by Sir Harold Gilles, used in 1942 to reconstruct the breast following mastectomy (2). The modern era of breast reconstruction was heralded by the use of tissue expanders (25,26) and the development of the silicone implant in 1963 (2,9,14,23) making single stage reconstruction a reality.

Autologous reconstruction sought to provide a more aesthetic and natural alternative whilst avoiding the drawbacks of early breast implants such as capsular contracture, increased risk of infection and rupture and their incompatibility with radiotherapy (27-29). Following

Figure 1 Breast cancer and reconstruction timeline.
refinement of the abdominoplasty technique in the 1950s, the rectus abdominis myocutaneous flap became one of the workhorse flaps in breast reconstruction (30). The pioneering work by Hartrampf et al. in the early 1980s saw the refinement of the transverse rectus abdominis myocutaneous (TRAM) flap (23,31-34), which along with advances in microsurgical technique meant the development of tissue donor sites and advanced flap variations (33). As techniques progressed, so to did the focus; minimising donor site morbidity and functional loss. In the late 1980s and early 1990s the musculocutaneous perforator flap (DIEP) described by Kroll, Rosenfield, Koshima and Soeda offered the benefits of TRAM and latissimus dorsi flaps with significantly reduced donor-site morbidity (23,36-38). The refinement of free flaps continued and as understanding of vascular territories, imaging technology and surgical expertise advanced, surgeons were demonstrating successful autologous reconstruction with minimal donor site morbidity using a variety of free flaps including superficial inferior epigastric artery (SIEA) (39,40), inferior gluteal artery perforator (IGAP) (41-47) and transverse upper gracilis (TUG) (48-50). The shift away from radical mastectomy to BCS (51) reflects in part the advancement of detection and effectiveness of chemo-radiotherapy, but also the changing perspective and expectation of patients. Although free-flap reconstruction remains the gold standard; donor site morbidity, lengthy operative time and microsurgical expertise means it remains costly and drives research into tissue-engineered autologous solutions. In Melbourne for example, researchers are working to achieve an autologous reconstructive option that eliminates the need for donor site morbidity by using a biodegradable chamber with vascularised adipose tissue at its core (NEOPEC) (52).

There is increasing interest worldwide in the use of adipose tissue as an autologous filler for breast defects following oncologic resection (53,54). Fat transfer has the advantage of using the patient’s own tissue with minimal donor-site morbidity and eliminating the issues of foreign body reaction or rejection associated with synthetic implants. In recent years, fat transfer has been gaining interest in the fields of breast and plastic surgery, not only due to volume replacement, but also the beneficial effects on irradiated tissue (55). Despite expansion of fat transfer techniques, understanding of the underlying mechanisms remains lacking. The ASAPS/ASPS position statement in 2012 (56) gives a succinct overview of the issues surrounding contemporary fat transfer and stem cell therapies, and acknowledges the need for increased efforts into both basic science investigations and translation into evidence based clinical therapies. When replacing volume, fat graft survival is one of the major problems, with long term follow up studies revealing 20–70% volume loss (57). The lack of revascularisation of grafts commonly underpins resorption, which is particularly problematic in breast reconstruction, where larger volumes of fat are required. The result is that multiple procedures are required, with increased direct and hidden financial and emotional costs. Several groups are looking into ways to overcome this problem (58). In this article we provide an overview of relevant adipose physiology, discuss aspects of cell biology and biomaterials relevant to adipose tissue regeneration, and highlight the potential risks and future potential avenues for research.

**Adipose physiology**

As a specialised thermogenic organ responsible for the regulation of metabolism and energy, adipose tissue forms an essential part of normal homeostasis (59). Originating from the mesoderm (60), the ratio of each adipose tissue type varies with age; brown adipose tissue (BAT), which is highly metabolically active, is found in greater quantities in infants and has an important role in maintaining core body temperature (59,61,62). Responsible for adaptive non-shivering thermogenesis, the expression of uncoupling protein 1 (UCP1) allows BAT to dissipate energy as heat in order to maintain temperature balance (60,63,64). The decline of BAT continues throughout infancy and adolescence; in adults it is virtually undetectable having been almost entirely replaced with white adipose tissue (WAT) (61). WAT, which develops in multiple anatomical sites, acts as a store for energy in the form of triglycerides (64), and as an endocrine organ releasing adipokines in response to physiological stimuli (65,66). White mature adipocytes, which are round or oval in shape, have a diameter of between 25–200 μm depending on location and, along with a few elongated mitochondria, contain a single lipid droplet (62,65). Occurring in two stages; the transition from an immature to a mature adipocyte starts initially by the determination and differentiation of a multipotent stem cell into an adipoblast and pre-adipocyte, followed by its terminal differentiation into a mature adipocyte (60). It is these mature white adipocytes that surgeons use to create autologous reconstructions either as lipoaspirate in a free-fat graft or as the volume component of a muscle free flap. Found within the stromal vascular fraction (SVF) are heterogeneous subpopulations of cells, growth factors and cytokines which
Contribute to the secretory function of adipose tissue (67-69). Enzymatic digestion of SVF liberates cells with multilineage potential; pericytes and supra-adventitial cells (CD34+/CD146+/CD45−) otherwise classified as adipose derived stem cells (ADSCs) (70).

Cell sources for adipose regeneration

As aforementioned, tissue engineering and stem cell research have the potential to revolutionise the future of reconstructive surgery by replacing tissue obviating need for donor site morbidity (71-73). Mesenchymal stem cells (MSC) are non-hematopoietic cells with multi-lineage potential and have been isolated from an increasingly varied number of tissues over the past 20 years (74). Having previously been isolated from umbilical cord blood, embryos and bone marrow, cells came at a high price and were often difficult to harvest and manipulate, and had variable quality as the cells aged (75-87). Balancing ease of harvest with yield and efficacy has been a delicate, and often difficult trade off which has prompted the scientific community to investigate alternative sources. In 2006, induced pluripotent stem cells (iPSC) were heralded as one potential solution; terminally differentiated cells were successfully regressed back into a state of pluripotency and demonstrated multilineage potential (88). Initially promising an abundance of easily accessible cells, the process of reprogramming the cells resulted in observed DNA errors, raising the question about the process and its effect on epigenetics (89,90). Focusing on the end tissue type, in 2002, researchers identified distinct stem cell populations within adult adipose tissue (91). ADSCs are multipotent MSC which exhibit characteristics similar to stem cells isolated from bone marrow stem cells (BMSC) (86,92-94). Compared to BMSCs however they are more readily accessible, easier to isolate and carry a significantly lower morbidity. Obtained from either excised fat or lipoaspirate (under local or general anaesthetic), adipose tissue yields between 100–1,000 times more stem cells per cubic centimetre than their bone marrow counterparts (95). The apparent abundance of these stem cell populations means relatively small reservoirs may have potentially significant yields of cells and therefore offer a reliable and easily accessible source of cells for tissue engineering (91,96,97). Moreover, ADSCs have a longer lifespan in culture than BMSCs prior to becoming senescent (94,98) which gives greater flexibility in the lab environment. ADSCs are naturally inclined to replenish lost volume by proliferation and maturation into adipocytes, which makes them ideally suited for adipose regeneration (99). However, identification of this stem cell population can pose a challenge; they are phenotypically very similar to MSCs of other origins and as such a combination of phenotype, morphology and secretory functions are required to distinguish this cell population as ADSCs (86,93,100,101). ADSCs have been shown to secrete several growth factors including vascular endothelial growth factor (VEGF), hepatocyte growth factor, FGF-2, and insulin-like growth factor 1 (IGF-1) which play a key role in angiogenesis and adipose tissue regeneration. This highlights the potential importance of ADSCs in maintenance of transplanted tissue volume, an important consideration in adipose tissue engineering (102). Identifying the appropriate cell type for the creation of de novo breast tissue is only part of the challenge; the cells will ultimately need a scaffold in order to create structure and stability while they mature and provide support when they are first implanted.

Scaffolds for adipose regeneration

Current approaches to soft tissue regeneration include the use of fat grafts, natural or synthetic biomaterials to act as a filler material, and scaffolds to enable 2D and 3D cell culture and engineering of tissue. Biomaterials act as the biochemical and biophysical environment to tune the cell response for the specific tissue engineering requirements (103). Whether derived from human, animal or naturally occurring sources, biomaterials by design have a tendency towards low immunogenicity and degradation or incorporation into recipient tissue (104). Focusing on ADSCs as a potential cell source for adipose tissue engineering; a number of studies have examined their interaction with natural and synthetic scaffolds, but there remains a paucity of literature on clinical application (103,105). The aim of the ideal scaffold is universal regardless of tissue type; to produce ‘native like tissue’ with equivalent physiological and biochemical structure (biomimetic). Minimising structural and functional deviation is key, so scaffolds must balance the structural integrity to withstand physiological forces while remaining flexible enough to allow ingrowth of new tissue and constructive remodelling (106). Immunological characteristics of scaffolds must also be considered, to prevent pro-inflammatory responses where possible (106,107). Natural biomaterials show good biocompatibility, degradability and the ability to support tissue regeneration; and while
synthetic materials are theorised to be more immunogenic, they have been shown to maintain mechanical integrity and assimilate over time (103,105).

Stem cell differentiation signalled by the extracellular microenvironment is a complex interplay of interactions, which is challenging to replicate (108). The material properties of potential scaffolds can influence lineage and differentiation as a result of their mechanical properties, mineralisation and chemical functionality (109). With the potential to function as extracellular matrix (ECM) in addition to supporting native ECM, biomaterials affect all facets of cell-scaffold interaction such as cell adhesion, proliferation and differentiation (110). The use of specific bioactive agents and material based delivery systems has developed the way in which scaffolds are viewed and our understanding regarding the control of stem cell differentiation and ultimate phenotype (111,112).

**Scaffold composition**

**Natural & biological scaffolds**

Natural materials explored to support adipogenesis include collagen, gelatine, silk and alginate (103,105,113-120). The application of silk, which is essentially a protein scaffold, has been extensively investigated across a number of engineered tissues. Silk fibrin demonstrates low immunogenicity and slow, controlled degradation, while maintaining adequate mechanical properties to allow cell seeding and new tissue formation, and has been shown to support adipogenesis in vitro and in vivo (103,105). Similar results were achieved in a comparable study, which examined the potential of lyophilised silk sponges and found they supported the adhesion of MSC in culture (121). Furthermore, the scaffolds allowed proliferation and infiltration of stem cells and supported remodelling when implanted in vivo (121). In keeping with providing patients with minimally invasive and low morbidity procedures, injectable scaffolds are gathering a greater research interest. Several biomaterial systems have been investigated to meet this clinical need; alginate/collagen microspheres, seeded with ADSCs are a promising injectable scaffold promoting the formation of fatty lobules after only 4 weeks in culture (122). A comparison of natural and synthetic hydrogels for use as an injectable scaffolds has been explored; alginate/o-carboxymethyl chitosan (O-CMC) and alginate/poly vinyl alcohol (PVA) with the inclusion of fibrin nanoparticles were compared (123). ADSCs demonstrated good adhesion, viability, proliferation and differentiation into adipocytes on these scaffolds. Cell differentiation studies of fibrin incorporated hydrogel scaffolds showed improved differentiation when compared to scaffolds without fibrin, which was confirmed by Oil Red O staining (123) (Table 1).

Biological scaffolds comprised of decellularised ECM are now widely used in preclinical and clinical tissue engineering studies (106). The preservation of structurally organised entities has been shown to act as a natural template and accommodate tissue regeneration (106); subcutaneous implantation of the adipose ECM in rats provoked a minimal inflammatory response and guided tissue remodelling and regeneration (126). Research evaluating the creation of more complex structures demonstrated that decellularised strategy for adipose tissue would provide a three-dimensional (3D) scaffold with adequate extracellular architecture (127). Seeding ADSCs on decellularised adipose tissue (DAT) demonstrated adipogenic differentiation, supporting the expression of the master regulators peroxisome-proliferator-activated receptor gamma (PPAR-γ2) and CCAAT/enhancer binding protein-alpha (CEBP-α), without the need for exogenous differentiation factors (127) (Table 2).

**Synthetic scaffolds**

Several synthetic polymers have been utilised for soft tissue regeneration including polyglycolic acid (PLGA), polyethylene glycol (PEG), polycaprolactone (PCL) and poly-l-lactic acid (PLA) (129-132). Fibrous scaffolds are a particularly promising type of scaffold due to their ability to mimic the native ECM environment and guide de novo tissue formation (133). Freshly isolated ADSCs demonstrated adipogenic differentiation on polypropylene fibrous scaffolds within 19 days, with the expression of adipogenic marker PPARγ2 (133). Similarly electrospun nanofibrous scaffolds made of PLA maintained adipogenic differentiation of human BMSCs (133). PLGA fibres seeded with human MSC (hMSCs) encapsulated within alginate/chitosan hydrogel capsules showed adipogenic differentiation and maintenance of the adipogenic phenotype for 56 days in immunodeficient mice (129). When evaluating efficacy however, few studies have directly compared natural and synthetic materials for soft tissue regeneration. Examining the commercially available scaffolds currently on the market; type I collagen sponge, PLGA and hyaluronic acid gels were compared for their suitability for adipose tissue engineering (108). Each were harvested and examined...
Table 1 Natural scaffolds

<table>
<thead>
<tr>
<th>Scaffold material</th>
<th>Derivation/synthesis</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silk</td>
<td>Protein polymers spun by lepidoptera larvae</td>
<td>Low immunogenicity; Slow and controlled degradation; Licensed for clinical use; Support adipogenesis <em>in vitro</em> and <em>in vivo</em> (124)</td>
<td>No long term data on stability of degradation products; Further work needed to allow surface modification</td>
</tr>
<tr>
<td>Collagen</td>
<td>Protein which is the main extracellular matrix component of most tissues</td>
<td>Can be modified e.g., addition of growth factors; Licensed for clinical use; Easily conjugated to other scaffolds; Improves ADSC differentiation and mature fat tissue formation <em>in vivo</em> (116)</td>
<td>Rapid degradation; Limited mechanical strength</td>
</tr>
<tr>
<td>Gelatin</td>
<td>A mixture of protein and peptides derived from collagen</td>
<td>Non-toxic; Enable rapid delivery of growth factors; Comes in various forms including hydrogel and microspheres; In combination with hyaluronic acid/collagen supports adipose tissue engineering (125)</td>
<td>Rapid degradation; Limited mechanical strength; For optimal results requires use in combination with another scaffold</td>
</tr>
<tr>
<td>Alginate</td>
<td>Polysaccharide derived from seaweed</td>
<td>Non-toxic; Licensed for clinical use in dressings; Can be a hydrogel or microsphere; Easy modification with growth factors or combination with other scaffolds; Alginate/collagen microsphere scaffolds allowed formation of fatty lobules by ADSCs (122)</td>
<td>Rapid degradation; Limited mechanical strength</td>
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</table>

Table 2 Biological scaffold

<table>
<thead>
<tr>
<th>Scaffold material</th>
<th>Derivation/synthesis</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological (Decellularised matrix)</td>
<td>Generated through decellularization of tissue to obtain extracellular matrix</td>
<td>Widely used in preclinical and clinical tissue engineering studies; Minimal inflammatory response; Allows preservation of structurally organized entities; Acts as a natural template to guide tissue remodeling and regeneration; Used to form mature adipocyte groups resembling native fat tissue (128)</td>
<td>Lengthy decellularization process; Risk of immunogenicity if not completely decellularised; Not easily mass-scaled as requires donor tissue of the exact size and shape</td>
</tr>
</tbody>
</table>

histologically and immunohistochemically after 4 and 8 weeks of implantation in athymic mice (108) (Table 3).

**Current scaffolds**

Of the readily available scaffolds, collagen sponges were found to be more suitable, having a higher expression of PPAR-γ2 and type I collagen than other two scaffolds. When combined with gelatin (collagen/gelatin mix), sponges modified with ADSCs and impregnated with basic fibroblast growth factor (bFGF) successfully demonstrated newly formed adipose tissue (125). Beyond promotion of adipogenesis, effects at the cellular level need to be taken into consideration; comparing natural fibrin glue with synthetic PCL as a framework demonstrates these differences in both cell yield and cell expression (136). After 6 weeks *in vivo* post seeding with ADSCs, adipose tissue and expression of adipogenic genes on the PCL scaffolds was significantly greater when
compared to the fibrin scaffolds (136). These smaller studies demonstrate there is a broad variation in scaffold composition and structure, which can have a significant impact on tissue growth and quality. Production of large volume engineered adipose tissue extends beyond the scaffold material; it is essential that the graft maintain its size, shape and volume over time post implantation. Vascularisation is a common barrier discussed in research papers and as the pro-angiogenic properties of ADSCs have been demonstrated, supporting new vessel formation will be an essential for any scaffold using this cell type (138,139) (Table 4).

### Scaffold free

The need for a scaffold has not been universally acknowledged however; research strategies examining scaffold free cell delivery is essentially an area that utilises one of three main techniques; use of single cells, cell sheet and micro tissues. Cell micro tissues have the advantage that they promote cell-to-cell and cell-to-matrix interactions (140) which is important in the formation of new tissue structure. Studies have demonstrated the ability of ADSCs to aggregate in cell culture and subsequently differentiate toward multiple cell lineage (140). It has been demonstrated that human ADSCs isolated from adipose tissue could be expanded and used to produce a 3D scaffold free micro tissue (141,142). The cells show uniformly positive expression for stem cell markers CD34, CD73, CD90, and CD105 are negative for CD19, CD14, and CD45, and were functionally inducible into adipocytes in appropriate medium (141). With the aim of enhancing adipogenesis by using a controlled delivery system; research has also explored the encapsulation of adipogenic factors within PLGA microspheres to deliver targeted growth within a transplanted fat graft (143,144). While this research is promising, translational barriers including scale-up limitations have led to an increasing

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**Table 3 Synthetic scaffolds**

<table>
<thead>
<tr>
<th>Scaffold material</th>
<th>Derivation/synthesis</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA Polyester porous scaffold</td>
<td>Biodegradable; Easily mass produced; Reproducible fabrication protocols; Can be modified with addition of motifs/groups or altering topography to improve cell adhesions/proliferation/differentiation; Promotes adipogenic differentiation of ADSC in vivo (134)</td>
<td>Adverse effects of degradation products (inflammation); Requires surface modification to optimize cell growth and differentiation</td>
<td></td>
</tr>
<tr>
<td>PEG Polyether compound</td>
<td>Licensed for medical use in laxatives and commercially in cosmetic products; Reproducible fabrication protocols; Low toxicity; Water soluble and biodegradable; Enzymatically degradable PEG-based gels promote formation of adipose tissue-like structures (135)</td>
<td>Does not provide mechanical strength; Requires conjugation to improve properties as a scaffold; Rapid degradation</td>
<td></td>
</tr>
<tr>
<td>PCL Biodegradable polymer</td>
<td>Reproducible fabrication protocols; Can be modified with addition of motifs/groups or altering topography to improve cell adhesions/proliferation/differentiation; Adds mechanical strength; Promotes ADSC adipogenesis in vivo (136)</td>
<td>Potentially unstable degradation</td>
<td></td>
</tr>
<tr>
<td>PLA Biodegradable thermoplastic polymer</td>
<td>Reproducible fabrication protocols; Easy surface modification; Adds mechanical strength; Maintains adipogenic differentiation of human BMSCs (137)</td>
<td>Rapid degradation</td>
<td></td>
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</tbody>
</table>

PLGA, polyglycolic acid; ADSC, adipose derived stem cell; PEG, polyethylene glycol; PCL, polycaprolactone; PLA, poly-l-lactic acid; BMSCs, bone marrow derived stem cells.
interest in bioreactors and associated technologies to form controlled 3D assembly of stable adipose tissue (141).

**Potential risks of and future challenges**

Seen now as a reliable technique for autologous correction of volume loss and contour defects (145), stem cell populations within WAT have been shown to hold significant regenerative potential (73,146-148). This has resulted in the refinement of techniques which aim to increase the concentration of ADSCs in order to optimise graft retention and maximise therapeutic benefit (146,148). However it is the properties that make ADSCs so desirable which present the greatest concern because the cell characteristics useful for tissue regeneration and wound healing are shared with those features capable of promoting tumour growth and progression (148,149).

As the risk of dormant cells or remnant cancer post mastectomy or BCS cannot be entirely excluded, the oncological safety of tissue-engineered therapies must be appropriately considered. Recently there have been a number of clinical trials examining the safety and efficacy of ADSCs and related therapies in an attempt to evaluate their clinical potential (150,151). Cancer proliferation and progression relies on several factors to regulate and structure the tumour microenvironment (152). ADSCs demonstrate the production of factors that promote vascularisation, tissue growth, immune-modulation and cell recruitment (149).

The expression of IL4, IL10, matrix metalloproteinase and SDF-1, in addition to pro-inflammatory mediators, has been shown to produce a microenvironment conducive to breast cancer recurrence (153). Additionally, the chemo-attraction of endothelial cells by production of VEGF and adipokines creates a microenvironment that can facilitate increased vascularisation, implicated in increased local recurrence risk (154). Conversely, the production of transforming growth factor (TGF) beta 1 and beta 2 has been shown to negatively affect cell differentiation and proliferation of breast cancer (155,156) demonstrating that ADSCs are only one part of a complex microenvironment. Increased metastatic potential has been further demonstrated in a study examining the co-culture of triple negative breast cancer and ADSCs; organ metastases were observed in the murine model in the ADSC group compared with none in the control (157).

The risk of de novo breast cancer is even less clear; the inhibition of hydrogen peroxide-induced cell death may play a factor in cell resistance to apoptosis and their propensity to develop down a carcinogenic line, however this hasn’t yet been fully demonstrated (158). While in vivo and in vitro studies have previously demonstrated the proliferative potential of ADSCs in the breast cancer environment (159); a recent meta-analysis of 2,428 patients found no significant difference in recurrence rates in the patient group who

### Table 4 Hybrid/injectable scaffolds

<table>
<thead>
<tr>
<th>Scaffold material</th>
<th>Derivation/synthesis</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-CMC</td>
<td>Injectable biodegradable cross-linked hydrogel</td>
<td>Reproducible fabrication protocols; Can mimic extracellular matrix; Uniform distribution when injected into tissues; ADSCs demonstrated good adhesion, viability, proliferation and differentiation into adipocytes(123)</td>
<td>Rapid degradation; Lack of mechanical strength</td>
</tr>
<tr>
<td>PLGA/hydrogel</td>
<td>Biodegradable hydrogel/polymer composite</td>
<td>Reproducible fabrication protocols; Increases mechanical strength whilst mimicking ECM; Encapsulating cells in fibrin/chitosan prior to seeding onto PLGA scaffold promotes adipogenic differentiation and maintenance of the adipogenesis (129)</td>
<td>Rapid degradation</td>
</tr>
<tr>
<td>Alginate/PVA with the inclusion of fibrin nanoparticles</td>
<td>Crosslinked scaffold</td>
<td>Can be a hydrogel or hollowfibre porous scaffold; Reproducible fabrication protocols; Increases mechanical strength whilst mimicking ECM; Incorporation of Fibrin into biocomposite scaffold improves adipogenic differentiation of ADSCs (123)</td>
<td>Unstable/uncontrollable degradation</td>
</tr>
</tbody>
</table>

O-CMC, o-carboxymethyl chitosan; ADSC, adipose derived stem cell; ECM, extracellular matrix; PVA, poly vinyl alcohol.
underwent fat grafting when compared to controls (160). Within this analysis however there was a single paper, which focused on in situ recurrence alone and found that the risk was 6 times greater in the group receiving fat grafts (161). Concerns regarding detection following breast surgery and fat grafting have been around since the late 1980s, and a number of studies have shown that there is no significant effect on radiographic surveillance following use of fat grafting (162). A retrospective study performed in the US found that all surgical procedures of the breast carried an increased risk of mammographic changes (163) and radiographic changes should be viewed in the context of patient specific factors.

Beyond the potential carcinogenesis risk, the complications of breast reconstruction must be taken into account. Issues with graft survival, volume loss and fat necrosis need to be considered. A systematic review found that 15.6% of patients reported a complication unrelated to recurrence demonstrating that non-malignant adverse events were not to be underestimated (164). Given the importance of reconstruction and follow-up in this patient group, it is essential that further studies examining both, the safety and efficacy of tissue engineered solutions, as well as effects on long term radiological monitoring, are carried out. It is only with further thorough, systematic studies, at a cellular and clinical level, that the true role of ADSCs in adipose tissue regeneration and cancer biology will be understood.

**Summary**

Surgeons and scientists face continued challenges in the coming years, to not only develop comparable and sustainable tissue engineered solutions with minimal donor site morbidity, but to prove their safety and efficacy. As the challenge to protect and improve patient quality of life continues to drive innovation forward, we cannot forget that these patients carry an inherent risk of potentially unseen or dormant breast cancer cells. The regenerative potential of ADSCs to generate de novo adipose tissue to replace lost breast volume has been well documented and is too good an opportunity to ignore. However, the use of stem cell therapies to expand and grow tissue for reconstruction must occur in the context of risk management. At each stage of clinical evaluation, patients must be fully informed of the benefits and potential risks. The autocrine and paracrine effects ADSCs must be fully investigated in rigorous clinical trials to evaluate their safety; and for those patients who have already undergone fat grafting following BCS, long-term follow up and careful monitoring is essential to examine the clinical impact of these therapies.

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**Footnote**

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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