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Cell sheets in cell therapies

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Abstract

This review aims to provide a broad introduction to the use of cell sheets and the role of materials in the delivery of cell sheets to patients within a clinical setting. Traditionally, cells sheets have been, and currently are, fabricated using established and accepted cell culture methods within standard formats (e.g., petri dishes) utilizing biological substrates. Synthetic surfaces provide a far more versatile system for culturing and delivering cell sheets. This has the potential to positively affect quality, and efficient, localized cell delivery has a significant impact on patient outcome and on the overall cost of goods. We highlight current applications of these advanced carriers and future applications of these surfaces and cell sheets with an emphasis both on clinical use and regulatory requirements.

Key Words: *culture, plasma polymerization, regeneration, sheet, therapy*

Introduction: why cell sheets

Cell therapies (CT) have historically been delivered by injection, transfusion or direct application, with the cells of interest in suspension. One of the first cell therapy (CT) approaches reported in the literature was a transfusion of sheep blood into a human in 1667 [1]. Later examples include in the 19th century when Charles-Edouard Brown-Sequard injected rat testicle extract into humans, attempting to stop aging [2]. Although some of these early interventions were of dubious merit, in 1968 the first successful bone marrow transplant was conducted [3]. In recent years, CT has evolved rapidly to the point where there are many cell types of interest for a wide range of indications [4]; however, these delivery routes have limitations, including a lack of control as to where the cells go and a non-homogeneous distribution of the cells within the treated area. Injection and transfusion are not necessarily best suited where localized delivery is necessitated. Therefore, biomaterials are significant as delivery devices for cell therapies [5].

The human body is full of planar membranes such as the skin, esophagus, uterus and bladder. For example, skin, as the largest organ in the body and the most accessible tissue, has been the subject of some of the earliest interventions. Skin grafting is thought to have originated in India approximately 2500 to 3000 years ago [6], with the concept gradually migrating into Western medicine. Bunger, a German physician, reported the successful transfer of skin from the buttock to the nose in 1869 [7], and in 1875, Wolfe reported the first full-thickness skin graft [8]. Many of these planar surfaces are difficult, if not impossible, to treat effectively with cell suspensions. Furthermore, the importance of cell-cell interactions may be critical to efficacy and modes of action, but as soon as adherent cells are placed into a suspension, this behavior is compromised, potentially affecting viability and function. Cell sheets negate this risk and may be used in some cases to deliver a denser cell population to the required site. By integrating the process of expansion, storage, transportation and clinical cell delivery, it would be possible to get the cell product to the patient more quickly, negating the risks associated with dense cell populations (loss of phenotype/differentiation).

A well-tested method for delivery of cell therapies to wound sites is outlined in Figure 1, demonstrating the extraction of healthy cells from the patient, their expansion *in vitro* and delivery to the wound site. This review focuses simply on planar surfaces for the delivery of cell monolayers and the application of materials

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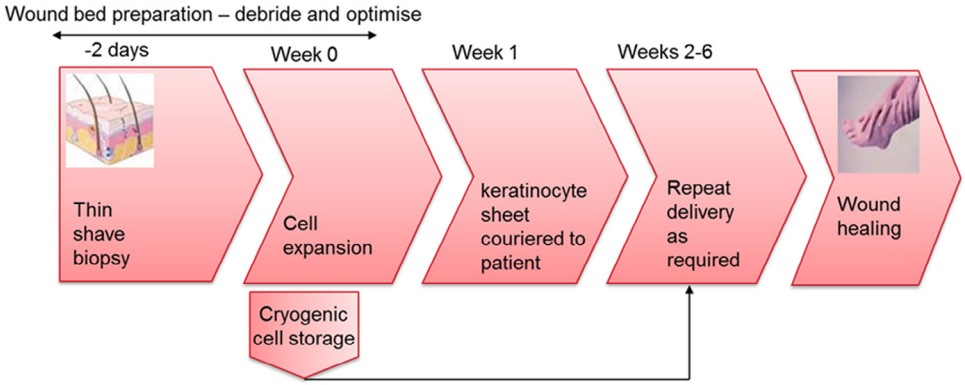

Figure 1. Simplified steps in the delivery of autologous cell therapies for wound healing.

science to optimize patient outcomes. In this review, we are defining cell sheets as cells that are either self-supporting or that are delivered from a supporting material but where the material plays no long-term role in the therapy. Cell sheets can be single or multiple layered, and even subconfluent in some cases. Various culture and release methods are used to generate cell sheets, with perhaps the most common being dispase (or other enzymatic) treatment [9]; other more exotic methods include thermo-responsive polymers [10], a range of inorganic complexes that respond to magnetic force [11], electrochemical polarization [12] and pH change [13]. However, each of these technologies relies on multiple steps and a high degree of manual handling or automation [14].

Society is on the cusp of a CT medical revolution; however, there are numerous challenges to overcome to deliver on the promise of CT. The quote (often attributed to Albert Einstein) "Make things as simple as possible, but not simpler" is a good philosophy for the development of cheap and effective clinical CTs, and there is a growing body of evidence suggesting that certain clinical indications are most suited to the delivery of two-dimensional monolayer/multilayer of adherent cells or sheets of cells. This includes resurfacing lost epithelial tissue [15], the cornea [16], esophageal [17], bladder [18], retinal [19,20], tracheal [21], uterine [22] and myocardial [23].

Although the approach of using cell sheets is highly suited to autologous CT, there is a corresponding effort to develop allogeneic (and cryopreserved) cell sheets [24]. A review for cell sheet development and emerging cell sheet technologies and applications is timely given the high academic and commercial interest in cell sheets.

The value of cell sheets has been identified commercially, and multiple companies are beginning to provide cell sheet products (Table I).

Historical development of biological substrates

Modern clinical use of intact cell sheets is built on the foundations of cell culture. The use of cultured autologous keratinocytes to treat burns is now a well-established clinical procedure [25–27]. The earliest confluent cell sheet therapies were developed as an extension to skin grafts for the treatment of extensive burns [28]. Those that demonstrated long-term effectiveness utilize autologous keratinocytes [29–35] and were based on (at the time) pioneering cell culture techniques. In 1975, Reinwald and Green first demonstrated the isolation and serial culture of human

Table I. Commercial entities that have invested in the future of cell sheets.

Company	Product(s)	Brief description
Japan Tissue Engineering Corp (J-TEC). Gamagori City, Japan	Autologous cultured epidermis	Cultured epidermal autograft
Japan Tissue Engineering Corp (J-TEC). Gamagori City, Japan	Autologous cultured corneal epithelium	Autologous corneal epithelial cells cultured on hydrogel
CellSeed, Tokyo, Japan	UpCell RepCell	Cultureware based on the poly(N-isopropylacrylamide) thermo-responsive polymer
Vericel, Cambridge, Massachusetts, USA	Epicel	Cultured epidermal autograft
Modex Therapeutics, Lausanne, Switzerland	Epidex	Autologous follicle keratinocytes attached to a silicone sheet (with 1% agarose)
Organogenesis, Canton, Massachusetts, USA	Apligraf	Bovine collagen and fibroblasts topped with keratinocytes
Organogenesis, Canton, Massachusetts, USA	Dermagraft	Polyglactin mesh with keratinocytes