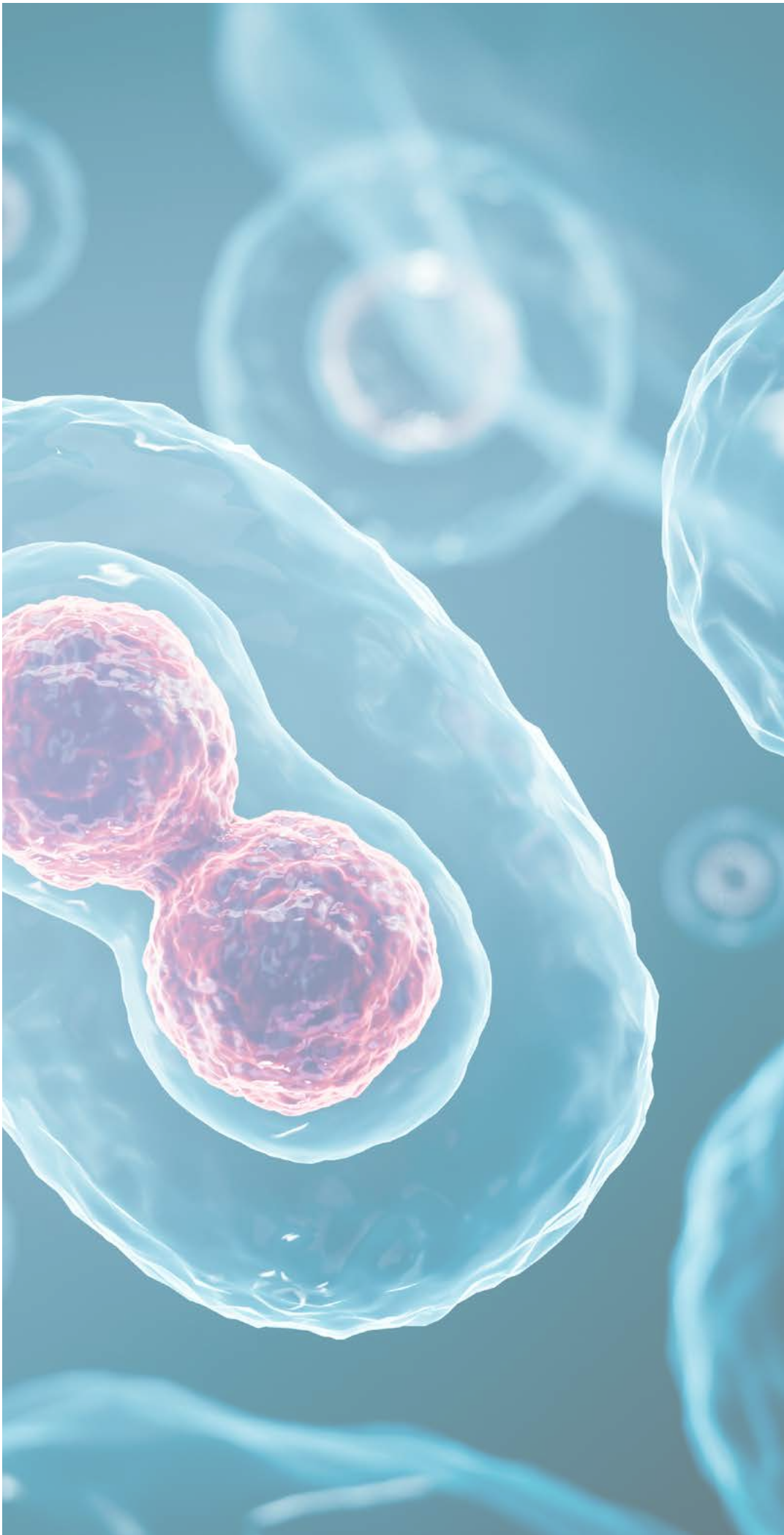


White paper



Cell delivery: The relevance of hydrogels.



Cell therapies, which use cultivated or modified cells to treat various diseases, are gaining attention from the pharmaceutical industry and regulatory authorities. Many of these cell therapies target cancers, particularly blood cancers. The success in this area has encouraged pharmaceutical companies to broaden their development of cell therapies for conditions that require locoregional cell delivery, like spinal cord injuries, chronic heart failure, or knee cartilage defects. However, locoregional

delivery of cells faces several challenges, including cell sedimentation during transport, shear stress during administration, cell viability and cell retention at the injection site. The use of hydrogels – such as collagen, gelatin, fibrin, hyaluronic acid and poloxamer - can mitigate these challenges. Rousselot Biomedical offers customizable solutions that meet regulatory standards for clinical use and can enhance the outcome of cell therapies.

The promise of cells as medicine

Cell therapy defined

Cell therapies use cells derived from patients or donors to treat various medical conditions, including solid tumors, hematological malignancies and neurological disorders. Cell therapy aims to treat diseases by introducing cells into the body to restore or alter specific cell sets or functions. To achieve this, cells are cultivated and may be genetically modified outside the body before they are administered. The cells can be autologous (sourced from the patient) or allogeneic (sourced from a donor). In the European Union, cell therapy products are regulated by the European Medicines Agency under Regulation (EC) No 1394/2007 on advanced therapy medicinal products (See text box 1).

According to **Regulation (EC) No 1394/2007**, Advanced Therapy Medicinal Products (ATMPs) are medicines for human use that are based on genes, cells or tissues. They are classified into three main types:

1. **Gene therapy medicinal products:** These involve inserting recombinant genes into the body to treat or prevent diseases.
 2. **Somatic-cell therapy medicinal products:** These use cells that have been manipulated to change their biological characteristics or are used for a different essential function in the body.
 3. **Tissue-engineered products:** These contain cells or tissues that have been modified to repair, regenerate or replace human tissue.
- The regulation also includes combined ATMPs, which integrate one or more medical devices as part of the therapy, such as cells embedded in a biodegradable scaffold

In the US, cell therapies are regulated under 21 CFR 600 – 680 through the Biologics License Applications process. The process is overseen by the Center for

Biologics Evaluation and Research (CBER), a division of the United States Food and Drug Administration (FDA) which regulates biological products such as vaccines, blood products, gene therapies and human tissues.

Cell therapy is on the rise

Cell therapy has gained significant attention from both the pharmaceutical industry and regulatory authorities. This is evident from the growing interest and investment in this field as well as the orphan drug designations and fast-track statuses that have been granted to certain therapies. To date, at least 32 gene therapies (including genetically modified cell therapy) and 68 non-genetically modified cell therapies have been approved for clinical use globally (ASGCT and Norstella, 2024). As of early 2024, another 2,881 therapies are in development, ranging from the preclinical phase to the pre-registration phase. In the first quarter of 2024 alone, 61 gene therapy and 42 non-genetically modified cell therapy clinical trials were initiated. A search of the clinicaltrials.gov database shows that the number of interventional studies filtered by “cell therapy” as an intervention or treatment has steadily increased over the past decade (Figure 1).

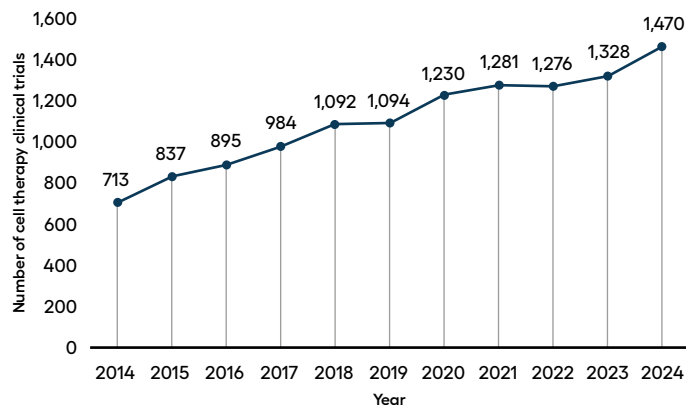


Figure 1. Number of interventional clinical trials with start date from 2014 to 2024, filtered by “Cell therapy” as an intervention or treatment, registered at clinicaltrials.gov.

Cell therapy targets

Oncology and rare diseases are the leading therapeutic areas for both approved products and those in the pipeline. For example, more than half of the gene and genetically modified cell therapies in the pipeline are forms of CAR-T cell therapy, with 97% targeting cancer indications (ASGCT and Norstella, 2024). CAR-T cell therapy involves collecting a patient’s own T cells and genetically modifying them to produce special receptors called chimeric antigen receptors (CARs). Once these CAR-T cells are infused back into the patient’s bloodstream, they can recognize and destroy cancer cells.

However, there is a growing interest in non-oncology targets for cell therapy. This is reflected in the increasing proportion of gene therapy clinical trials focusing on non-oncology indications (ASGCT and Norstella, 2024) (Figure 2). As of December 2022, 59.8% of active cell and gene therapy clinical trials still targeted cancer, but other areas were also being explored. These included cardiovascular (6.2%), dermatology (4.7%), neurology (4.3%), infections (4.0%) and musculoskeletal (3.7%) indications (Cell & Gene, 2023).

In addition, of the 61 gene therapy clinical trials initiated in the first quarter of 2024, 43% targeted non-oncology indications. Similarly, 55% of the 42 clinical trials for non-genetically modified cell therapies focused on non-oncology indications. The most common non-oncological diseases targeted by non-genetically modified cell therapies in the pipeline include Parkinson’s, arthritis and type 1 diabetes (ASGCT and Norstella, 2024).

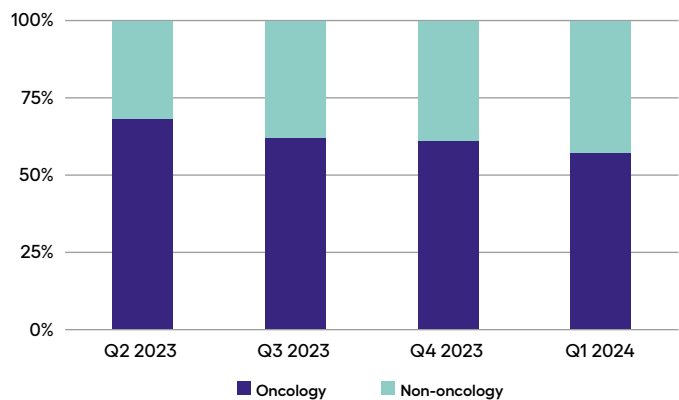


Figure 2. Proportion of gene therapy clinical trials with and without oncology indications. Data source: ASGCT and Norstella (2024).

Delivering therapeutic cells to the target

Locoregional cell delivery

Most currently approved cell therapies involve the infusion of cells directly into the patient’s bloodstream using an intravenous cannula. However, a considerable number of cell therapy targets like solid tumors, chronic heart failure or knee cartilage defects, require locoregional delivery of cells (Table 1). This means the therapeutic cells need to be delivered in or at a specific location in the body, through routes other than infusion into the bloodstream. This locoregional delivery allows the cells to engraft directly at the site of injury or disease.

Locoregional cell delivery is achieved by injecting cells directly into the affected area using a syringe filled with cells suspended in a gel or culture medium. In other currently approved products, cells from patients or donors are cultured on a matrix or scaffold (Figure 3), which is then transplanted into the patient. With the increasing focus on non-hematological targets of cell therapy, locoregional delivery will become increasingly relevant for future cell therapy products.

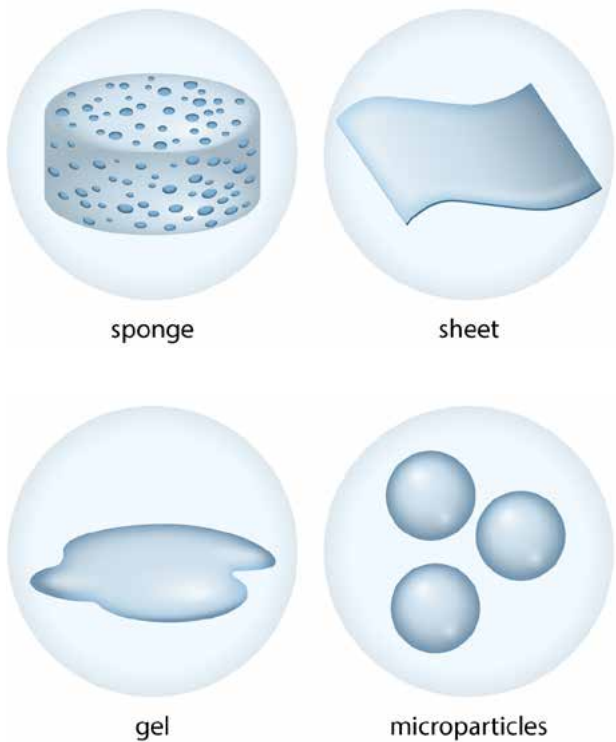


Figure 3. Examples of matrices or scaffolds to be transplanted in patients

Table 1. Locoregionally administered cell therapy products with current marketing authorization. The data is sourced from information available through relevant regulatory agencies and the product's company websites.

Name	Generic name and description	Site of delivery	Year of approval	Region of approval	Indication	Cell delivery vehicle
Apligraf™	Bilayer skin equivalent consisting of allogeneic neonatal dermal fibroblasts and keratinocytes	Ulcers and wounds	2000	USA	Treatment of diabetic foot and venous leg ulcers	Bovine type I collagen matrix
Chondron™	Cultured autologous chondrocytes	Knee cartilage	2001	South Korea	Treatment of focal cartilage defect of knee	Fibrin
Holoderm®	Autologous skin-derived keratinocytes	Skin	2002	South Korea	Treatment of 2nd and 3rd degree burns	Cultured cell sheet.
Kaloderm®	Allogeneic keratinocytes (cell sheet)	Skin	2005	South Korea	Treatment of 2nd degree burns (2005) and diabetic foot ulcer (2010)	Cultured cell sheet.
KeraHeal®	Autologous skin-derived keratinocytes	Skin	2006	South Korea	Treatment of 2nd and 3rd degree burns	Phenol red-free high-glucose DMEM
CaReS®	Autologous cartilage cells	Cartilage	2007	EU	Treatment of articular surface defects	Collagen type I matrix
JACE®	Human autologous epidermis-derived cell sheet	Skin	2007	Japan	Treatment of burns (2007), giant congenital melanocytic nevus (2016), dystrophic epidermolysis bullosa and junctional epidermolysis bullosa (2018)	Cell sheet cultured on extracellular matrix (ECM) produced by feeder cells
RMS Ossron™	Cultured autologous osteoblasts	Bone marrow	2009	South Korea	Focal bone formation	Fibrin
CureSkin Inj	Autologous dermal fibroblasts	Intradermal	2010	South Korea	Treatment of depressed acne scar	Serum-free cell medium
Queencell®	Autologous adipose tissue-derived adipose cell	Subcutaneous	2010	South Korea	Improvement of subcutaneous fat defect	DMEM
laViv®	Azficel-T, autologous cellular product	Nasolabial fold wrinkles	2011	USA	Improvement of the appearance of moderate to severe nasolabial fold wrinkles in adults	Phenol red-free DMEM
Cartistem®	Human Umbilical Cord Blood-	Knee cartilage	2012	South Korea	Treatment of knee articular cartilage defects in patients with osteoarthritis	Phenol-red free MEM alpha, Sodium hyaluronate

Cupistem®	Anterogen, autologous adipose tissue-derived mesenchymal stem cells	Rectal fistula	2012	South Korea	Treatment of fistula in patients with Crohn's disease	DMEM
Gintuit	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen sheet	Surgical created vascular wound bed	2012	USA	Treatment of mucogingival conditions	Bovine collagen
JACC®	Autologous chondrocytes	Knee cartilage	2012	Japan	Treatment of traumatic cartilage defect or osteochondritis dissecans (excluding gonarthrosis) of the knee	Atellocollagen gel
Neuronata-R®	Lenzumestrocel, autologous bone marrow mesenchymal stem cells	Spinal theca	2014	South Korea	Treatment of amyotrophic lateral sclerosis	Autologous cerebral spinal fluid
Novocart 3D	Human articular chondrocytes on collagen matrix	Knee cartilage	2014	Switzerland	Biological reconstruction of articular cartilage defects of the knee joint	Collagen scaffold
HeartSheet	Human autologous skeletal myoblast-derived cell sheet	Heart	2015	Japan	Treatment of serious heart failure caused by ischemic heart disease	Cultured cell sheet. Unknown if an ECM is used, but collagenase is used in the manufacturing process
Holoclar	Ex-vivo expanded autologous human corneal epithelial cells containing stem cells	Cornea	2015	EU	Treatment of adult patients with limbal stem cell deficiency	Fibrin membrane
KeraHeal-Allo™	Allogeneic skin-derived keratinocytes suspended in a thermosensitive hydrogel	Skin	2015	South Korea	Treatment of 2nd degree burns	Phenol red-free high glucose DMEM with 26% poloxamer 407
MACI	Autologous cultured chondrocytes on porcine collagen membrane	Knee cartilage	2016	USA	Repair of symptomatic, single or multiple full-thickness cartilage defects of the knee	Porcine collagen
Ortho-ACI™	Autologous cultured	Knee, patella and ankle cartilage	2017	Australia	Treatment of cartilage lesions associated with the knee, patella and ankle	Collagen patch
Rosmir®	Injectable autologous fibroblasts	Nasojugal groove	2017	South Korea	Improvement of nasojugal groove	Phenol red-free DMEM

Spherox	Spheroids of human autologous matrix-associated chondrocytes	Knee cartilage	2017	EU	Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee	Isotonic sodium chloride solution
			2018	Switzerland		
Alofisel®	Darvadstrocel, allogeneic fat tissue-derived mesenchymal stem cells	Rectal fistula	2018	EU	Treatment of complex perianal fistulas in adult patients with non-active/mildly active luminal Crohn's disease	DMEM with HSA
			2021	Japan		
Nepic®	Human autologous corneal limbus-derived corneal epithelial cell sheet	Eye surface	2020	Japan	Corneal epithelium reconstruction in patients with limbal stem cell deficiency	Cell sheet cultured on ECM produced by 3T3-J2 feeder cells
Ocural®	Human autologous oral mucosa-derived epithelial cell sheet	Ocular surface	2021	Japan	Repair of corneal epithelium defects in patients with limbal stem cell deficiency	Cell sheet cultured on ECM produced by feeder cells
Rhetymic®	Allogeneic processed thymus tissue	Quadriceps muscle	2021	USA	Immune reconstitution in pediatric patients with congenital athymia	Cultured cell sheet
StrataGraft®	Allogeneic cultured keratinocytes and dermal fibroblasts in murine collagen	Wound bed	2021	USA	Treatment of thermal burns	Murine collagen
Sakracy	Human autologous oral mucosa-derived epithelial cell sheet using a human amniotic membrane substrate	Ocular surface	2022	Japan	alleviation of adhesions on the ocular surface in patients with limbal stem cell deficiency	Human allogeneic amniotic membrane
Jacemin	Melanocyte-containing human autologous epidermis-derived cell sheet	Skin	2023	Japan	Re-pigmentation of vitiligo patients by supplying epidermal cells containing melanocytes	Cell sheet cultured on ECM produced by 3T3-J2 feeder cells
Vyznova	Neltependocel, fully differentiated allogeneic human corneal endothelial cells	Anterior chamber cornea	2023	Japan	Reconstruction of damaged corneal endothelium monolayer tissue in patients with bullous keratopathy	Cultured cell sheet. Unknown if an ECM is used, but bovine collagenase is used in the manufacturing process
Cellspan Esophageal Implant™	Autologous adipose-derived mesenchymal stromal cells on a polyurethane tubular mesh	Esophagus	Orphan disease application in 2023	EU	Esophageal reconstruction	Polyurethan tubular mesh

Intravenous versus locoregional delivery of cells

Systemic, intravenous delivery of therapeutic cells has shown impressive clinical responses in treating certain types of blood-related cancers (Stern & Stern, 2021). However, there are still limitations of systemic cell therapy, both for cancer and non-cancer targets. When cells are administered in the bloodstream, a large number of cells is required to ensure enough cells reach the intended target site. The complexity and resource intensity involved in developing these cells can make systemic cell delivery quite costly (Jørgensen et al., 2020). In addition, therapeutic cells circulating in the bloodstream may inadvertently

target healthy tissues, potentially leading to severe side effects and limiting the administration of sufficient cells to achieve the desired therapeutic effect (Williams et al., 2016).

Therefore, particularly in cases of localized injuries or diseases, or when tumors have not metastasized, directly delivering therapeutic cells to the affected area can help overcome some of these challenges. This locoregional approach allows for a higher concentration of cells at the target site, resulting in greater efficacy with fewer cells, reduced systemic side effects, and lower cost.

The challenges of locoregional delivery of cells

Locoregional delivery of cells comes with its own set of challenges. For instance, the volume—and therefore the number of cells—that can be injected at the target site is limited although the number of therapeutic cells that reach the target site is still typically higher than with systemic delivery (Gualeni et al., 2018). Other common issues related to locoregional cell delivery are cell sedimentation during transport, inconsistent delivery of therapeutic cells to the target site, poor retention of the cells at the target site and maintaining high cell viability from start to finish of the cell therapy process (Amer et al., 2017).

Injection volume and cell number

Little research exists on the optimal injection volume for therapeutic cell delivery. However, exceeding the tissue's capacity during injection can result in tissue damage. While increasing the cell concentration may seem like a solution, high cell concentrations in suspensions can lead to challenges such as needle clogging, uneven injection flow and limited oxygen and nutrient diffusion, which may cause cell death. Additionally, administering large local doses of cells poses safety risks, including tumor formation and micro-embolism (Amer et al., 2017).

Cell sedimentation

During the transportation of therapeutic cells from the production site to the patient, cells tend to sediment in the vial or syringe (Figure 4). This not only affects cell viability but also causes inconsistent dosing (Torres et al., 2015). For example, when syringes are stored or transported vertically, cells clumping together at the bottom of the cannula can lead to most cells being delivered early on, while in horizontally stored

syringes, cells settle on the side of the cannula and are delivered toward the end. Non-uniform cell doses can create hotspots of therapeutic cells that could cause serious adverse effects, like the graft-induced dyskinesias observed in Parkinson's disease trials (Ma et al., 2002).

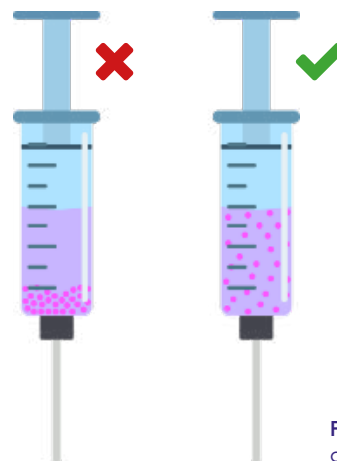


Figure 4. Potential behavior of cells in syringe

Shear-stress-induced damage

Cells can be damaged when they are forced through a needle (Figure 5), which affects their ability to survive and function after transplantation (Aguado et al., 2012). The mechanical forces on the cell surface as it flows through the confined space of a needle are called shear stress. Shear stress significantly affects cells, with even low levels leading to the activation of molecular cascades, altering cell viability and function. In addition, when cells are clumped together, shear stress may increase, potentially destroying cells.

Cell retention at the site of injection

One of the significant challenges in local or regional

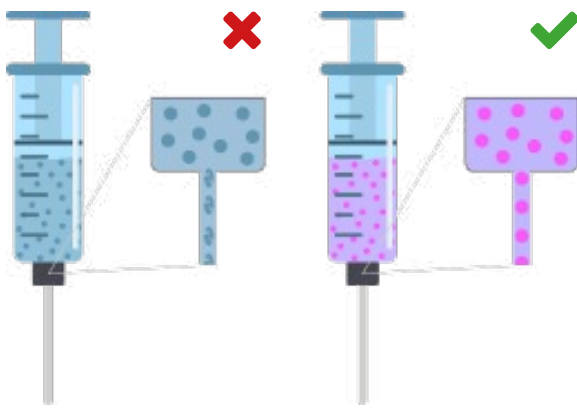


Figure 5. Shear stress of cells when forced through a needle

cell therapy is ensuring that therapeutic cells stay at the injection site to exert their intended effects (Figure 6). The fate of the cells after injection is often unknown, but studies have shown that less than 5% of injected cells remain at the target site shortly after injection (Amer et al., 2017). This low retention rate can be attributed to various factors. Blood vessels or lymphatic vessels could carry cells away, tissue pressure can squeeze the cells out or the cell suspension can flow back via the needle access point.

Cell viability post-injection

Even if sufficient viable cells have been delivered at the target site, cell death may still occur. In contrast to cells delivered through intravenous

injection, locoregionally delivered cells that rely on the local blood supply of their oxygen and nutrients may die due to inadequate vascularization in the surrounding tissue. In addition, cells may undergo apoptosis or another form of programmed cell death (anoikis) due to a lack of an anchorage-dependent survival signal from the surrounding extracellular matrix. This signal is generally provided by so-called RGD cues, a specific sequence of amino acids - arginine (R), glycine (G) and aspartic acid (D) that is commonly found in proteins which facilitate cell adhesion. Furthermore, the injection site is often an inflammatory microenvironment where macrophages and other immune system-related processes may remove transplanted cells (Abdelwahid et al., 2016). The release of intracellular contents of dying (therapeutic) cells may in itself further trigger an inflammation response in the tissue (Park et al., 2023).

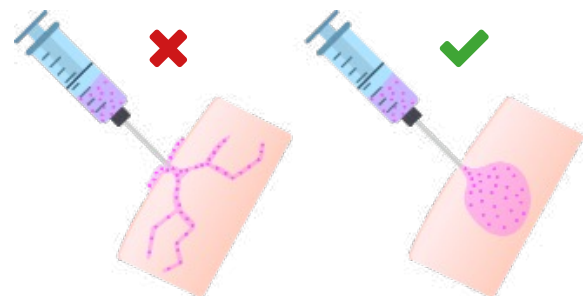


Figure 6. Cell retention at injection site

Solutions for locoregional delivery of cells

Researchers in the field of cell therapy, like Professor Dr. Mangala Srinivas, Chair Group Leader at Wageningen University & Research, in Cell Biology and Immunology, are continuously working to overcome challenges related to the locoregional delivery of therapeutic cells, such as optimizing cell viability and migration and developing methods to track the cells after injection.

Optimizing the therapeutic dosing regimen

Effective cell therapy requires finding the optimal balance between delivering a sufficient number of therapeutic cells to achieve the desired effect while avoiding excessive cell death or tissue damage.

According to Professor Dr Srinivas, "In contrast to intravenous cell delivery, the number of cells that can be administered in a single locoregional injection is often restricted by the small surface area of the therapeutic target area."

As shown in her own work, simply increasing the cell concentration in a single injection is not always

beneficial. This approach carries risks of needle clogging and limited nutrition for the cells, and it can also change their behavior. Using radioactive (¹¹¹In)-labeled dendritic cell vaccines Professor Dr. Srinivas' group demonstrated that varying the number of injected cells affected their intended migration to nearby lymph nodes (Aarntzen et al., 2013).

Researchers are exploring various strategies to optimize the delivery of viable cells to the target site. These strategies include administering multiple simultaneous injections, adjusting needle sizes or using designs such as hollow microneedle devices (Gualeni et al., 2018).

To monitor the behavior and viability of the cells post-injection, researchers have various tracking options (Helfer et al., 2021; Srinivas et al., 2010). Next to radioactive labeling, one approach involves labelling the cells with small, superparamagnetic iron particles, which can then be tracked over time using magnetic resonance imaging (MRI) (de Vries et al., 2005).

"Understanding the fate of the cells after injection

is of paramount importance for optimizing the therapeutic dosing regimen”, Professor Dr. Srinivas states. Information on the fate of transplanted cells is also of interest to regulators responsible for approving new cell therapeutic products.

The use of hydrogels

Research has demonstrated that some challenges of locoregional delivery of cells can be overcome by the use of hydrogels (Kharbikar et al., 2022). Using hydrogels to carry the cells during transportation can help maintain a uniform suspension (Aguado et al., 2012). Shear-thinning hydrogels can also reduce shear stress, leading to a more even distribution of mechanical forces on the cells as they pass through an injection device. Furthermore, hydrogels can act as physical barriers, preventing direct contact between the transplanted cells and the immune cells or their anti-inflammatory molecules. For example, a shear-thinning hydrogel can (temporarily) prevent the cells from flowing away, and from being recognized by the immune system, thereby protecting the cells from being scavenged by macrophages (Qi et al., 2015). Furthermore, cell adhesion and differentiation is facilitated with hydrogels that contain RGD cues

and other biochemical signals. Hydrogels can also provide a 3D scaffold that mimics the extracellular matrix, providing mechanical support (Riha et al., 2021). According to Professor Dr. Srinivas, “Physical factors such as shear stress and mechanical support have so far not received much attention in research aimed at optimizing therapeutic cell delivery. Next to exploring various therapeutic cell types and the impact of biochemical factors, further investigation into physical factors could play a significant role in enhancing the overall success of cell therapy.” In summary, locoregional delivery of therapeutic cells presents several challenges. One significant hurdle is maintaining high cell viability, which can impact both the effectiveness and safety of cell therapy. Selecting the appropriate type of therapeutic cell is crucial, as different cell types exhibit various sensitivities to factors such as shear stress and inflammatory environments. Tracking the fate of the cells after injection can help monitor their viability and optimize the dosing regimen. Additionally, using hydrogels as an injection vehicle or scaffolding material can protect the cells from both biochemical and physical stressors.

Types of hydrogels used in combination with therapeutic cells

Hydrogels are versatile materials that are attractive to use as a biomaterial in combination with therapeutic cells (Lopez Hernandez et al., 2021). Certain biopolymers, like gelatin, collagen and fibrin, primarily form solid hydrogels. Others, like poloxamer, usually behave more like liquids (Abou-Shamat et al., 2019). The physical state of a hydrogel—whether solid or liquid—can be influenced by factors such as temperature, pH or pressure, depending on the specific type of hydrogel. Because of the nature of the molecular interactions, these biomaterials can

transition from a viscous liquid solution into a solid hydrogel. Such biopolymer-based solutions can be used for many cell therapy applications, including the formation of (temporary) scaffolds or as carriers in therapeutic cell delivery. Various hydrogels are available to combine with therapeutic cells, both from natural or synthetic sources or a combination thereof (Keshavarz et al., 2024). The most commonly used hydrogels in currently approved cell therapy products include collagen, gelatin, fibrin, hyaluronic acid and poloxamer (Table 2).

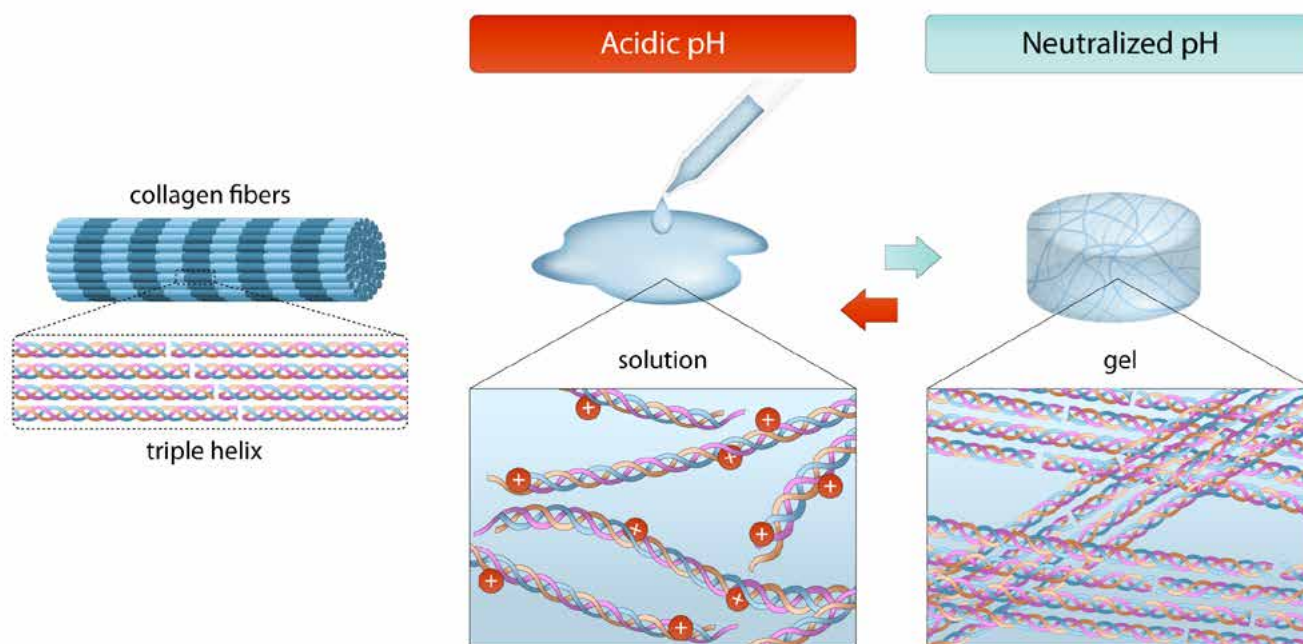
Table 2. Properties of different hydrogels used in approved cell therapy products

	Natural or Synthetic	RGD cues	Prevention of sedimentation	Protects against shear stress	Helps with cell retention	Supports cell viability post-injection	Reference
Fibrin	Natural	Yes	No	Yes	Moderate	Yes	Martens et al., (2009)
Collagen	Natural	Yes	No	Yes	Moderate	Yes	Awonusi et al., (2023)
Gelatin	Natural	Yes	Yes	Yes	Minimal	Minimal	Sisso et al. (2020)
Gelatin microparticles	Natural	Yes	Yes	Yes	Yes	Yes	Feng et al., (2024); Shen et al., (2024)
Hyaluronic acid	Natural	No	No	Yes	Yes	Moderate	Kaleka et al., (2018)
Poloxamer	Synthetic	No	No	Minimal	Moderate	No	Khattak et al., (2005); Lippens et al., (2013)

Collagen and gelatin

Collagen is the most prevalent protein in the human body, constituting approximately 30% of total protein content. It provides strength, elasticity and structure to various tissues, including skin, bones, cartilage, tendons, ligaments and blood vessels. The use of collagen as a biomaterial is popular because of its excellent physicochemical and biocompatible properties (Larue et al., 2024). Common sources of collagen and its partially hydrolyzed form, gelatin,

used as biomaterials are bovine and porcine origins. The formation of collagen hydrogel can be controlled by adjusting the pH and temperature. In acidic conditions, collagen becomes positively charged, causing the collagen fibrils to fall apart into individual positively charged triple helix domains. When the pH is neutralized, these triple helix domains self-assemble back into fibrils resulting in the formation of a solid collagen gel. This fibrillogenesis process is slow and can be further delayed by cooling.



For the preparation of collagen as a cell delivery vehicle, an acid-soluble collagen solution is neutralized and mixed with cells. During the neutralization, the solution is kept cool to drastically slow down fibrillogenesis. Once the fluid is injected into the body, it heats up, and fibrillogenesis spontaneously occurs, forming a solid gel.

Gelatin is produced from collagen through the partial hydrolysis of the peptide bonds in its triple helix structure. This process causes the collagen to unravel, forming long, polypeptide gelatin chains. The reduction in molecular weight and the unravelling of the triple helix domain allow the material to dissolve in water at physiological pH. When cooled, the triple helix domains partially restore, leading to intense hydrogen bonding and the formation of a solid hydrogel. Increasing the temperature above the melting point will reverse the gelation.

Gelatin has been used in the medical field for decades and comes in various forms. Its first application in medical formulations was as a stabilizer for vaccines containing enveloped live attenuated viral antigens. Gelatin is well-documented for protecting cell membranes during storage and injection and can prevent cell sedimentation through its thermoreversible gelation behavior. Gelatin can also

be transformed into a shear-thinning gel. A shear-thinning gel based on gelatin microparticles typically exist in a solid state under ambient conditions. However, when shear is applied to the gel—such as when it is pushed through a needle—it transitions to a liquid-like state. Once the gel is injected and the shear is relieved, it returns to its solid state (Pitenis et al., 2023) (Figure 8).

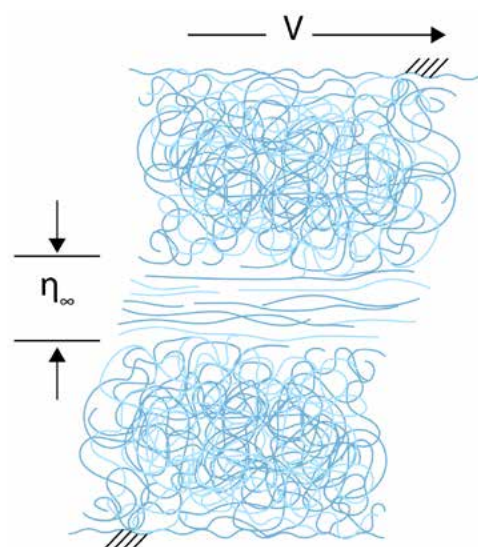


Figure 8. Representation of the relationship between shear and viscosity. Based on Pitenis et al. (2023).

Fibrin

Fibrin is a protein that naturally occurs in blood plasma and is essential for blood clotting. When a blood vessel is injured, a solid fibrin clot is formed from the soluble protein fibrinogen through the action of an enzyme called thrombin. The fibrin creates a mesh-like structure that serves as a temporary seal over the wound. Once the tissue has healed sufficiently, the fibrin seal is broken down and removed from the body through a process called fibrinolysis.

The mesh-forming properties and biocompatibility of fibrin also make it valuable as a biomaterial. Fibrin is obtained by mixing two solutions, one containing fibrinogen and the other containing thrombin. When these two components are combined, soluble fibrinogen and thrombin react to spontaneously form fibrin polymers: clots. Consequently, fibrin can act as a sealant (glue) during surgery to help reduce local bleeding; it is applied by dripping or spraying it onto the wound (Goczyńska et al., 2021). Fibrin is also used as a scaffold material or cell delivery vehicle for wound or cartilage repair (Bayer, 2022; Rojas-Murillo et al., 2022). When using fibrin as a cell delivery vehicle with syringes, it is important to ensure that its immediate clot-forming properties do not result in clogged needles.

Hyaluronic acid

Hyaluronic acid is a naturally occurring polysaccharide, found mainly in the skin, connective tissues and eyes. Its negative charge allows it to bind a large amount of water, resulting in a highly viscous gel. In the body, this gel not only moisturizes the skin but also offers lubrication in the joints and acts as a shock absorber (Sze et al., 2016).

Due to its excellent biocompatibility, moisture retention, and viscoelasticity properties, hyaluronic acid is a popular component of medical devices. For example, the US-FDA has approved hyaluronic acid injections in knee joints for pain relief in patients with osteoarthritis (Walker et al., 2023). Also, hyaluronic

acid is very popular in the field of dermal fillers. However, as hyaluronic acid breaks down in the body, the volume that is created upon injection diminishes over time.

In addition to application as fillers, hyaluronic acid is also used as biomaterial for delivering therapeutic cells. For example, a cell therapy product approved in Korea uses hyaluronic acid to deliver mesenchymal stem cells to treat knee cartilage defects (Ghisa & Zaslav, 2024). Hyaluronic acid is produced by bio fermentation using recombinant bacteria.

Poloxamer

Poloxamer is a synthetic block copolymer used in pharmaceuticals and cosmetics. It is composed of three blocks: two hydrophilic polyethylene oxide blocks flanking a central hydrophobic polypropylene oxide block. This structure gives it unique properties, making it useful as a stabilizer or emulsifier (Russo & Villa, 2019).

Poloxamers are thermosensitive: they remain fluid at room temperature and become viscous gels at near physiological temperatures. The properties of poloxamers can also be modified by changing the pH or salt concentration. Poloxamer gelation behavior triggered by heating of the fluid makes it highly interesting for locoregional delivery of cells and APIs. However, due to the gel transition at body temperature, poloxamer might block needles during injection. As a result, their use is mainly limited to topical administration and injections with short time windows (Chung et al., 2020; Khattak et al., 2005; Lisiecka, 2024).

Others

Other materials of interest for use as hydrogels include alginate and chitosan (Keshavarz et al., 2024). However, to date, these materials have not yet been included in clinically approved cell therapy products.

Selecting a suitable hydrogel to combine with therapeutic cells

There is no one-size-fits-all approach to selecting the most suitable hydrogel for therapeutic cell delivery. The ideal properties of the hydrogel will differ based on the intended use. In addition to the hydrogel's functionality, considerations should include the source and the necessary level of standardization.

The function of the hydrogel

When selecting a suitable hydrogel, the primary factor to consider is its functionality. For example, hydrogels used to reduce shear stress in cell delivery devices such as syringes or cannulas will require

different properties than those used as membranes to culture cells into tissue sheets. In addition to differences in viscosity between injectable cell vehicles and scaffold materials, the degradability of the hydrogel may need to be faster for those used only to deliver cells at a site of injury or disease compared to those that serve as support systems in the body for tissues that recover slowly. Hydrogels used as scaffolding materials may benefit from using biomaterials like collagen, gelatin and fibrin, which naturally provide RGD cues that help promote cell differentiation and attachment to surfaces. In certain cell therapy applications, the ideal hydrogel would not only reduce shear stress during cell delivery but also support cell growth at the injury site. In such cases, biomaterials with tunable properties may be the best option.

The source of the hydrogel

Most hydrogels used in currently approved cell therapy products are derived from natural sources. They are derived from human or animal tissues or produced by recombinant bacteria. A significant advantage of naturally occurring biomaterials - like collagen, gelatin, fibrin and hyaluronic acid - is that our bodies typically do not recognize them as foreign objects, unlike some synthetic materials. In addition, RGD cues from materials like gelatin and fibrin promote tissue regeneration in a way that mirrors natural processes in the human body. This is often difficult to replicate with synthetic materials. Natural hydrogels like collagen, gelatin and fibrin degrade by cell-mediated proteases such as matrix metalloproteinases (MMPs). This degradation process is much faster than hydrolysis processes

which dominate the degradation of synthetic materials. A fast degradation process can be a disadvantage when treating chronic conditions or for tissues that heal slowly. Nevertheless, like synthesized polymers, natural biomaterials can be modified to enhance specific physicochemical functions, such as degradation rate, adhesiveness and mechanical strength. A challenge with natural materials is the variability in the starting material. To achieve consistent clinical outcomes, it is crucial for any material to have consistent and stable material properties, which requires robust production processes and quality management systems.

Regulatory landscape

Although many types of biomaterials have been developed for delivering cells, only a few have been approved by regulatory agencies like the US FDA and the EMA (Table 1). Reasons for the relatively low number of approved products isn't fully clear, but could be related to a limited understanding of how they work, difficulties to comply to regulations or the manufacturing process being too complex and not cost-effective (De Pieri et al., 2021; Mandal et al., 2020). Especially for natural biomaterials, navigating the regulatory landscape can be challenging. Regulatory experts will require extensive documentation to ensure regulatory experts will require extensive documentation to ensure that these products do not trigger pyrogenic, immunogenic or toxic responses due to the presence of (biological) contaminants. Regulators will also demand the biomaterials to be of consistent, reliable and reproducible quality.

The benefits of Rousselot's gelatin in effective cell delivery

Gelatin has a long history of safe use in biomedical applications. Compared to synthetic hydrogel materials, gelatin is generally not recognized by the immune system as a foreign body. Rousselot Biomedical has developed gelatin-based solutions for locoregional therapeutic cell delivery. Gelatin has a high affinity for cell membranes and will protect cells from stress during storage and injection.

Additionally, Rousselot Biomedical can tune the biodegradation rate of the hydrogel to meet specific application needs, allowing it to degrade within hours or weeks after injection. Rousselot Biomedical offers ultrapure medical-grade gelatins that comply with the purity and regulatory requirements for use in clinical products.

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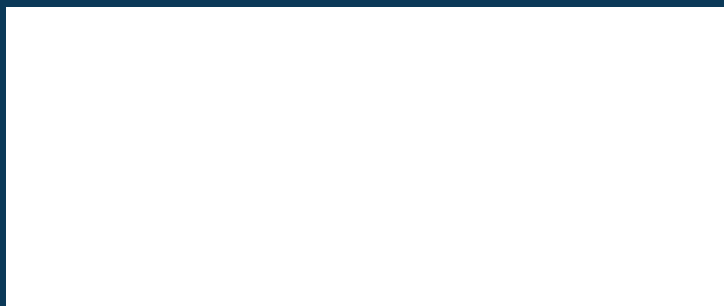


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