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CELLULAR REPROGRAMMING: AN APPROACH TO TISSUE ENGINEERING AND NANOTECHNOLOGY

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Abstract: In recent years, there has been a growing development of studies with gene editing, in particular, the process that involves cellular reprogramming. The development of techniques that sought to improve these methodologies was, and has been studied until today, as a way of advancing more and more in this research. This work seeks to make a compilation of recent works published in this area of bioengineering; making a historical overview of the techniques that were/are used in cellular reprogramming. As reprogramming factors, bioactives delivery systems, biochemical and biological mechanisms involved in the regulation of this process.
Keywords: Tissue engineering. Cell reprogramming. Epigenetics. Biomaterials. Nanotechnology.

INTRODUCTION

Stem cells, due to their relevant characteristics for regenerative biomedicine—such as a high capacity for differentiation and cell proliferation—have become the focus of various research lines aimed at treating numerous diseases. They offer hope for the potential cure of diseases with diverse levels of complexity, whether incurable or difficult to treat (KIRSCHSTEIN; SKIRBOLL, 2001; RAFF, 2003; SADEGHI 2024).

Their high differentiation capacity, known as pluripotency, is one of the most valuable characteristics for therapeutic purposes (NIH, 2006; YU et al., 2007; SHI, 2009; Xiong, X. 2024). Cellular differentiation is characterized by the series of stages that an immature cell undergoes until it becomes functional and specific to a particular tissue or organ. The more differentiated a cell is, the more specific and limited it becomes, committing only to acting in that particular tissue (ZATZ, 2004; KARP, 2005; NIU 2024).

Due to this commitment of differentiated cells, studies have sought to understand how this cellular process occurs and what is directly related to it. It was soon discovered that various transcription factors are directly involved in this process from embryonic development. This discovery paved the way for cellular reprogramming (TAKAHASHI; YAMANAKA, 2006; XIAO 2023).

Cellular reprogramming consists of the exogenous expression of genes with relevant roles during the embryonic period to restore the pluripotency of already differentiated cells. This process resulted in the so-called iPS—Induced Pluripotent Stem Cells. These cells have characteristics similar to stem cells, both in morphology and plasticity (THOMSON et al., 1998; TAKAHASHI et al., 2007; XIAO 2023).

The use of somatic cells to produce a genetically functional cell lineage embryonic-like stem cells has revolutionized many fundamental and translational research ideas (ROMANAZZO et al., 2020). Somatic cell reprogramming techniques create new opportunities for drug screening, disease modeling, artificial organ development, and cell therapy (KIM, JEONG, CHOI, 2020).

The ability to manipulate the induction and maintenance of cellular pluripotency is of great interest, as studying these processes related to early embryonic development can provide significant contributions both to its possible therapeutic use and to clarifications regarding nuclear remodeling, cellular commitment, and differentiation. Much research has been conducted and reviewed regarding the characteristics of pluripotent cells derived from mammalian embryos and the potential challenges of their use in medical practice. Despite being the focus of various studies, the mechanisms of their in vitro maintenance are still not fully understood (BRESSAN, 2013; CLEVELAND, 2024).

The interest in new research lines has led to the emergence of new experimental techniques. However, ethical and religious concerns regarding the therapeutic potential of stem cells are notable (BARTH, 2006; YU 2021). In this context, induced pluripotent stem cells (iPSs or iPSCs, from the English "Induced Pluripotent Stem Cells") were discovered. These are nothing more than genetically reprogrammed adult cells that regain the ability to differentiate into all cell types present in the body (Box 2 - iPSs) (LEVIN *et al*., 2019).

These cells were first generated in 2006 from mouse cells and, a year later, from human cells, marking a breakthrough in eliminating the ethical concerns associated with using embryonic stem cells. This is because iPSs do not require embryos, as they are obtained from the patient's own adult cells. Additionally, iPSs offer another advantage: they avoid immune rejection, which is the process in which the patient's immune system attacks and combats foreign cells, as commonly occurs when using cells that do not belong to the recipient (LEVIN *et al*., 2019).

Several pathways and reprogramming alternatives have been reported as possible candidates for use in reprogramming, including viral vectors, mRNA, miRNAs, episomal plasmids, oocyte proteins, and genetic signaling molecules (MALIK & RAO, 2013; WANG 2021). With the understanding of DNA as the basic unit of heredity, the ability to perform genome modifications has become a constant practice in modern medicine. This allows for the correction of altered genes or modifications at specific sites, enabling genetic improvements in patients with disorders at the DNA level (GOLÇALVES & PAIVA, 2017; YU 2021). Beyond reprogramming factors, selecting an appropriate transporter among various biological, physical, or chemical delivery systems is essential to enable and enhance cellular reprogramming. Undoubtedly, various drug and

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gene delivery strategies developed for genome editing, tissue engineering, and disease therapies can also be tested and applied for cellular reprogramming (FANG *et al*., 2020).

With the simultaneous development of nanomaterials and molecular biology, the bio-nano interface has introduced several applications of hybrid nanoparticles in nanomedicine. Hybrid nanoparticles not only retain the properties of their individual components but also exhibit synergistic effects for specialized applications. The development of hybrid nanoparticles for marking target cells and for diagnostics and therapies for various diseases has become of great interest in nanomedicine. Nanomaterials can be categorized based on different shapes, sizes, and components. By adjusting these aspects, new nanomaterial properties can be generated for specific applications. Integrating biological molecules with nanomaterials opens new prospects for nanotechnology in the future of medicine. Nanoparticles with suitable size and characteristics are being produced for systemic and intracellular delivery (D'ABREU, 2017; KO, 2020; ZHANG, 2022).

The application of nanotechnology in medicine emerges as a new interdisciplinary research field across various scientific domains, combining medical advancements with engineering. Its primary goal is to manufacture nanodevices and nanomaterials for biological applications (D'ABREU, 2017).

This review focuses on cellular reprogramming, exploring tissue engineering and nanotechnology while compiling promising studies on the subject. Here, we review current bibliographies on cellular reprogramming factors, delivery systems (physical, chemical, and biological) used in modulating cellular reprogramming, and nanoparticle systems.

LITERATURE REVIEW

TISSUE ENGINEERING

Cell reprogramming technology provides a revolutionary approach that bypasses traditional cellular and developmental biology rules in determining cell fate, enabling the conversion of mature somatic cells into pluripotent cells or other distinct cell lineages (Takahashi & Yamanaka, 2006; Yu, 2021).

Tissue engineering (TE) is a typical interdisciplinary research field that combines biology, biomaterials engineering, and medical technology to address tissue/organ damage, repair, regeneration, and replacement. Biomaterials often play a vital role in cell adhesion, proliferation, and extracellular matrix (ECM) secretion, and they can also serve as carriers for delivering appropriate growth factors or cytokines to aid in tissue repair and regeneration. Therefore, the careful selection of biomaterial components and structures is the foundation of tissue engineering (Jin et al., 2020).

Regenerative medicine has become one of the most significant research topics in medical science since it was first introduced by Leland Kaiser in 1992 (Kaiser, 1992; Niu, 2024). As defined by Mason and Dunnill, it refers to the "process of replacing, engineering, or regenerating human cells, tissues, or organs to restore or establish normal function." This field has evolved into an interdisciplinary area that includes tissue engineering, gene therapy, biomaterials, nanotechnology, and many other disciplines. Among these, nanomaterials and nanotechnology have rapidly developed and are widely applied in various fields, including electronics, medical devices, optics, and medicine. In recent years, the application of nanomaterials and nanotechnology in regenerative medicine and tissue engineering has gained widespread research interest (Zheng et al., 2021).

The biomaterials sector has been a growing interdisciplinary field in recent decades, driven by its vast potential applications in wound healing and disease treatment. In tissue engineering, biomaterials are used in implants or to restore damaged tissue in injured areas (Costa & Barbosa, 2021).

Waste-derived biomaterials have been widely used in tissue engineering due to their non-toxic degradation properties, biocompatibility, and broad selection of renewable sources (Bee & Hammid, 2020). The clean synthesis of biomaterials with excellent physicochemical properties has led to the production of waste-derived materials for tissue engineering (Las Heras et al., 2020). In addition to their commercial value and environmental benefits, waste-derived materials offer specific advantages for the regenerative and replacement aspects of tissue engineering (Zamri et al., 2021). For example, chitosan is easily extracted from seashells and shrimp shells, which are typically discarded as waste (Zamri et al., 2021).

Notably, the physicochemical properties of biomaterials can be tailored through advanced chemistry and micro/nanoprocessing techniques, not only for delivering reprogramming factors but also as adjustable physicochemical cues in two-dimensional (2D) substrates or three-dimensional (3D) microenvironments (Crowder, 2016; Zhang, 2022).

CELL REPROGRAMMING

Pluripotent stem cells can self-renew and differentiate into cells from all three embryonic germ layers as well as germline cells. These characteristics drive numerous studies on these cells, as their versatility allows for various applications in animal production and human and veterinary regenerative therapy (De Paepe et al., 2014; Arnhold & Wenisch, 2015; Ko, 2020).

Natural and synthetic biomaterials have the capacity to interact and coordinate with biological systems. When performing their functions, they must be safe and non-harmful to the patient. In the field of regenerative cell therapy, regulating cell fate, especially concerning stem cells and the reprogramming of mature cells, is crucial. The reprogramming process must be precisely monitored and controlled; otherwise, if left unsupervised, it could result in the formation of teratomas instead of providing a therapeutic solution for the patient (Angelo et al., 2019).

Since its discovery, direct reprogramming has emerged as a significant process for managing cell fate. The overexpression of various factors, either general reprogramming factors or lineage-specific transcription factors, is responsible for altering the fate of already differentiated cells. In this context, biomaterials can provide biochemical signals or physical and topographical stimuli to cells, guiding and influencing the reprogramming of cell fate (Kim & Tae, 2016; Xie, 2024).

Indirect reprogramming has also been demonstrated as an alternative approach. This process involves converting somatic cells through a transient induction into a partially reprogrammed state using Yamanaka factors, followed by differentiation into the target cell type (Lujan & Wernig, 2013; Huang, 2024).

Pioneering studies in 2006 reported the induction of already differentiated murine cells into a pluripotent state (Takahashi & Yamanaka, 2006). This reprogramming was achieved through the introduction of exogenous genes that expressed transcription factors associated with embryonic pluripotency and cell proliferation. These *in vitro* reprogrammed cells are known as iPSCs (induced pluripotent stem cells), and in 2007, the same research group reported the production of human iPS cells (Takahashi et al., 2007). The *in vitro* genetic induction of somatic cells into a plu-

ripotent state allows to produce autologous pluripotent cells without passing through the embryonic stage, thereby eliminating both rejection issues and ethical concerns.

The generation of porcine iPS cells (piPSCs, or porcine-induced pluripotent stem cells) offers significant advantages for both translational research in regenerative medicine and animal production. Studies have already demonstrated the differentiation of piPSCs into cardiomyocytes (Montserrat et al., 2011), hepatocytes (Ao et al., 2014), neuronal lineages (Dolezalova et al., 2014; Kim et al., 2019; Machado et al., 2020b), and ocular tissue (Niu et al., 2024). The production of piPSCs has been reported in at least 25 studies, with only one utilizing a non-integrative methodology that resulted in iPS-like porcine cells. However, none of these studies used cells collected through minimally invasive or non-invasive methods for reprogramming (Pessôa, Bressan & Freude, 2019).

Cell reprogramming depends on a variety of factors responsible for its process. Throughout its development, multiple reprogramming factors have been used in research involving this technique, many of which are genetic regulators (Recchia, 2020). The most commonly employed factors in scientific research include DNA, plasmid DNA (pDNA), messenger RNA (mRNA), microRNA (miRNA), and the CRISPR-Cas9 system (Figure 1) (Black, 2018; Weltner, 2018; Roloff, 2019; Wang, 2020).

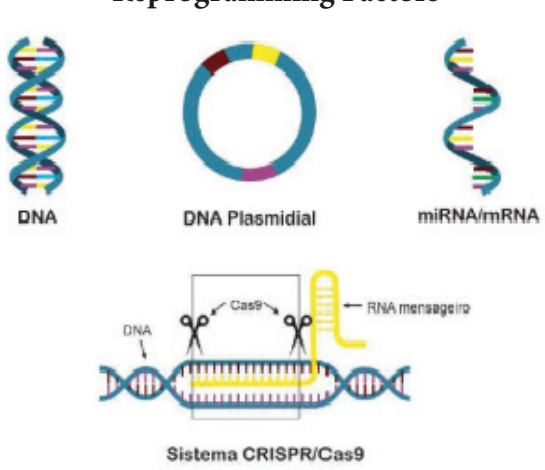

Figure 1: The main factors used in cellular reprogramming. Image adapted from FANG et al., (2020).

The CRISPR system (Clustered Regularly Interspaced Short Palindromic Repeats) is a highly innovative tool for genetic editing, with applications across various scientific research fields (Wang et al., 2020). The impact of this tool has led to significant technological advancements, particularly in treating diseases that previously only had palliative care options (Wilson et al., 2018). Its prominence in recent decades stems from its ability to address genetic defects even before the onset of disease (Hussain, 2019; Khadempar, 2019).

The origin of the CRISPR-Cas system can be traced back to an immune defense mechanism in bacteria and other microorganisms. Over evolutionary time, these organisms developed CRISPR-Cas as a means of protecting themselves against foreign nucleic acids from other species, which could potentially be harmful to their own genome (Barrangou, 2007; Zhang et al., 2021). In subsequent studies, various scientists hypothesized and formulated how CRISPR-Cas could be used for genetic editing until they established the foundations of the tool as we know it today (Ishino, 1987; Jasen, 2002; Mojica, 2005; Jinek, 2012; Bassett, 2014; Haapaniemi, 2018; Sen, 2024).

Further studies on the CRISPR-Cas system demonstrated that Cas9 protein could serve as an alternative for gene editing when combined with a specific CRISPR RNA, working alongside a trans-activating CRISPR RNA. This complex led to the development of the hybrid CRISPR/Cas9 system, which is currently the most widely used tool for genetic editing in both eukaryotic individuals and other species (Cong, 2013; Li JF, 2014; Ader, 2022).

Plasmid DNA (pDNA) is also used in cell reprogramming as a synthetic vector. It consists of a double-stranded circular DNA molecule containing regulatory gene sequences that mobilize the transcriptional and translational machinery of cells to enable protein expression (Crapina, 2019; Qin, 2024). This factor interacts with target cells in various ways, such as permanently deactivating a gene in the host genome, correcting or replacing a non--functional gene, or introducing a gene (or gene segment) for antigen/antibody expression in the target cell (Foldvari et al., 2016). pDNA has been widely used as a reprogramming factor due to several advantages: ease of production, manipulation, and storage, as well as its scalability for large-scale applications (Nafissi, 2014; Arjunan, 2024). However, one major disadvantage is the lack of a protective mechanism to shield the molecule from endonucleases that could degrade it. As a result, pDNA is either used infrequently or applied in well-regulated environments where endonucleases are absent, or with delivery systems that inhibit their activity (Yin et al., 2014).

Messenger RNA (mRNA) has also been explored as a possible factor in the cell reprogramming process. It emerged as an alternative to DNA-based gene therapy, offering some advantages, the most notable being reduced immunogenicity and a lower risk of mutations (Davis, 2009; Hudson, 2024). However, there are also disadvantages: mRNA cannot freely pass through the plasma membrane due to its large size, hydrophilicity, and negative charge. Additionally, mRNA is susceptible to degradation by endonucleases, requiring a delivery system that ensures targeted action at its specific site while minimizing non-specific interactions and rapid clearance (Kormann, 2011; Islam, 2024).

Synthetic microRNAs (miRNAs) are double-stranded RNA structures designed to integrate into an organism's RNA machinery, thereby exerting their function. Their potential lies in their ability to silence virtually any gene once introduced into a cell. However, their main disadvantage relates to their delivery system, which faces the same challenges as mRNA delivery (Ginn, 2013; Xiao, 2023).

DELIVERY SYSTEMS

For delivery factors to fulfill their role in the cell reprogramming process, they must reach their target site. Before reaching their destination, these reprogramming factors may be subjected to various agents that lead to their metabolism and eventual elimination. Therefore, delivery systems are necessary to ensure these factors are effectively transported to their site of action. Various delivery systems are involved in this process, including physical, chemical, and biological delivery methods (Modarai, 2018; Wu, 2019; Islam, 2024).

Among physical delivery systems, two main groups can be identified: electroporation and membrane deformation (Figure 2). Electroporation acts on the cell membrane by applying electrical pulses, leading to the temporary formation of nanopores that allow the delivery factor to enter the cell (Boukany, 2011; Yang, Q., 2024). Electroporation can be further divided into three categories: Bulk Electroporation (BEP) – This technique involves suspending cells in a conductive solution placed between two electrodes that emit electrical pulses. These pulses create nanopores, allowing material to enter the cell through

diffusion (Chang, 2016; Xiong, D., 2024). Although this method can be used to deliver a wide range of materials, its major drawback is the high level of cellular damage caused by the high voltage of the electrical pulses (Gallego-Pérez, 2017; Shi, 2018; Larouche, 2019).

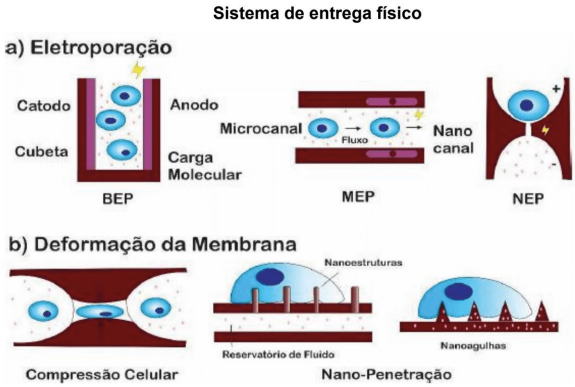

Figure 2: The two main types of physical delivery systems applied to cellular reprogramming. a) Subtypes of electroporation: bulk electroporation (BEP), microchannel electroporation (MEP), nanochannel electroporation (NEP). b) Membrane deformation delivery system through cell compression and nano-penetration. Image adapted from FANG et al., (2020).

The microchannel electroporation (MEP) system was developed to overcome the limitations of bulk electroporation (BEP), particularly the high voltage required for electrical pulses. MEP has increased both delivery efficiency and cell viability (Ding et al., 2017). However, its most significant advantage lies in the precise control of dosage, and more recently, a symmetric microscale electroporation system has been developed, integrating cameras to allow real-time visualization of reprogramming factor delivery (Yang et al., 2011). Despite these advantages, MEP still faces the limitation of cargo size, as the delivery process remains diffusion-based (Ouyang et al., 2017).

With advancements in bioengineering research, electroporation techniques have been further refined, leading to the development of the latest delivery system: nanoelectroporation (NEP). This highly efficient system utilizes optical tweezers for delivery. Compared to its predecessors, NEP provides a faster and more efficient delivery process, with precise dosing of reprogramming factors without compromising cell viability. However, a major drawback is that its scalability, delivering factors to multiple cells in a short time is challenging due to the labor-intensive and slow handling of optical tweezers (Ding, 2017; Cao, 2019).

Membrane deformation is another physical delivery method developed to introduce reprogramming factors into cells without exposing them to electric fields. This approach is particularly useful for cells that are difficult to transfect using the previously mentioned techniques. Membrane deformation can be achieved through **cell compression** or **nanostructures**. Where Cell Compression mechanically deforms the membrane, creating temporary pores that allow material entry. Its main advantages include the use of simple devices and rapid delivery. However, its major limitation is variability in cell size, which can affect the efficiency of delivery (Stewart et al., 2018). While nanostructures involve the use of nanoscale devices to facilitate the delivery of reprogramming factors. These structures can penetrate the cell membrane and introduce materials into various intracellular locations. Compared to cell compression, nanostructures offer higher efficiency and improved cell viability (Kollmannsperger, 2016; Xu, 2018; Chen, 2020).

Viral vectors were among the first methods developed for reprogramming factor delivery. This approach utilizes genetically modified viruses, including adenoviruses, Sendai viruses, and retroviruses, to introduce transcription factors into target cells using plasmids (Figure

3). However, several challenges are associated with viral delivery systems, such as immune responses and off-target effects in unintended cells, as well as limited packaging capacity due to the constraints of the viral envelope (Takahashi, 2016; Shi, 2017; Mangeot, 2019; Lyu et al., 2019; Tsukamoto, 2024).

Additionally, vector mobility remains a major concern for *in vivo* applications, as it can lead to various complications.

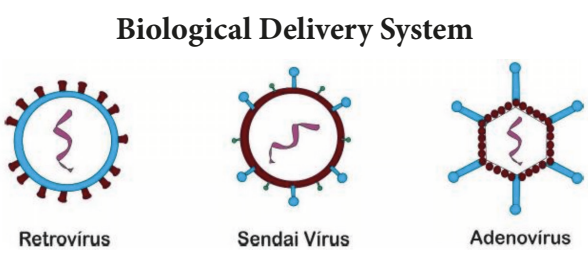

Figura 3: SIstema de entrega biológico, mostrando os principais vírus geneticamente modificados usados para transferência de DNA para as células. Imagem adaptada de FANG et al., (2020).

Han et al. (2012) compared a viral delivery system with a non-viral delivery system in the context of plasmid DNA delivery, specifically designed to encode a green fluorescent protein (GFP) reporter gene. Both delivery methods were administered via the subretinal route, and tissue analysis revealed that the viral delivery system had migrated beyond the retina, reaching the visual pathways in the brain. In contrast, the non-viral system remained confined within the retina. This finding highlights a critical safety concern regarding viral delivery, as its ability to migrate to unintended tissues can lead to unpredictable and adverse effects.

Despite various challenges, immunogenicity remains the primary concern associated with viral delivery systems. This issue arises because organisms naturally mount an immune response against viruses, complicating their use in *in vivo* applications. As a result, studies utilizing viral vectors often require immunosuppressants to achieve therapeutic effectiveness. Even with these measures, strict monitoring and the concurrent administration of antiviral drugs are necessary to control viral activity and minimize risks (Abou-El-Enein, 2014; Tsukamoto, 2024).

Chemical delivery systems rely on nanocarriers or nanoparticles to transport reprogramming factors. These systems offer a wide range of applications due to their ability to efficiently cross biological membranes and their large surface area for cargo loading. As a result, they are extensively used for the delivery of drugs and genes (Figure 4). The Key advantages of chemical delivery systems include its low immunogenicity, reducing the risk of immune rejection, minimal genetic integration, lowering the chances of unintended gene insertions and reduced risk of mutations at the target site, enhancing safety.

Currently, bioengineering research utilizes lipids, polymers, and hybrid systems as the foundational materials for these chemical delivery platforms (Xu, 2017; Behzadi, 2017; Wang, 2018; Tao, 2023).

Figure 4: Chemical delivery system, employing lipids, natural and synthetic polymers, as well as inorganic hybrids, image adapted from FANG et al., (2020).

BIOPHYSICAL AND BIOCHEMICAL REGULATION OF CELLULAR REPROGRAMMING

The manipulation of surface topography at the micro- and nanoscale has a profound influence on cellular functions, including morphology, adhesion, proliferation, and migration (Dalby, 2007; Wong, 2017). The topography of biomaterials, such as parallel microgrooves and aligned nanofibers, has been shown to significantly enhance the generation of induced pluripotent stem cells (iPSCs) by altering cell shape, which leads to increased histone acetylation and methylation and promotes the mesenchymal-to-epithelial transition (MET) (Downing, 2013; Li, Y. Y., 2024).

The underlying mechanisms of topography-induced effects on cellular reprogramming are not yet fully understood (Dalby et al., 2007). However, topography is strongly correlated with focal adhesion dynamics, which influence intracellular signaling pathways triggered by topographical parameters of substrates (Kulangara et al., 2014). Additionally, topography-induced cytoskeletal alterations can cause nuclear deformation, leading to changes in nuclear mechanics and chromatin state, ultimately affecting cell phenotype and function (Wang, 2016; Kim, 2024).

Substrate stiffness is one of the most widely applied physical cues used to modulate cell differentiation (Engler, 2006; Song, 2024). Extracellular mechanical forces play a crucial role in organ development and function in both health and disease, triggering cytoskeleton-mediated mechanotransduction and intracellular signaling cascades that regulate cellular reprogramming (Yang, 2011; Li, P. L., 2024).

Extracellular fluids - including blood plasma, interstitial fluid, and lymph - are critical for substance exchange and microenvironment maintenance. Fluid shear stress, an essential factor in liquid flow, can induce cytoskeletal reorganization, activate mechanosensitive gene expression, and alter cellular behavior, demonstrating a key role in vascular homeostasis (Yang, 2011; Umeyama, 2024).

In vivo mechanical stress and tension play fundamental roles in guiding cell behavior and tissue homeostasis. At the cellular level, mechanical stretching has been shown to regulate cell spreading, growth, lineage commitment, and stem cell differentiation in 2D substrates (Cui, 2015; Wang, 2024).

Additionally, mechanical stretching can be utilized for tissue regeneration in vivo, such as by activating capillary stem cells, recruiting macrophages, and promoting M2 phenotype polarization, which facilitates capillary regeneration in a tension- and duration-dependent manner. Recent studies have also shown that mechanical stretching positively influences reprogramming efficiency (Kim, 2017; Na, 2024).

Mechanical forces driven by electromagnetic or electric fields have been found to enhance cellular reprogramming. For example, extremely low-frequency electromagnetic fields significantly improved the efficiency of fibroblast reprogramming by dynamically regulating epigenetic modifications through the activation of a histone lysine methyltransferase (Baek, 2014; Sendera, 2024).

3D microenvironments provide biomimetic systems that can guide cell fate (Fatenullah, 2016; Liu, 2024). 3D biomaterial-based environments enable robust spatial and temporal control of biophysical and biochemical cues, which modulate cell fate both *in vitro* and *in vivo*. It has been demonstrated that mouse and human iPSC reprogramming can be enhanced using hydrogels (Caiazzo, 2016; Abuwatfa, 2024). This 3D approach led to a more than threefold increase in reprogramming efficiency compared to 2D environments, due to accelerated MET and enhanced epigenetic remodeling.

Organ-on-a-chip is an emerging technique and a powerful tool for disease modeling, drug discovery, toxicology research, and regenerative medicine (Zhang, 2019; Thenuwara, 2024).

NANOPARTICLES AND CELLULAR REPROGRAMMING

Nanotechnology is defined by the European Medicines Agency as the use of small structures with a diameter of less than 1,000 nanometers, designed to exhibit specific properties. Various nanoparticles are currently being developed for both therapeutic and diagnostic purposes (Lammers, T.; Aime, S., 2011; Fang, 2020).

The materials used in nanotechnological formulations are selected based on their biodegradability, biocompatibility, surface functionalization capacity, conjugation potential, complexation ability, and encapsulation properties (Moghimi, S. M., 2005; Yang, X., 2024). The main nanocarriers applied in therapeutics include liposomes, lipid nanoparticles, micelles, polymeric nanoparticles, and nanocrystals (Moghimi, S. M.; Hunter, A. C., 2005; Rahmat, 2024). Nanoparticle-based formulations can be used for drug delivery and production as well as in the manufacturing of biomaterials. Various cellular materials have been studied using both experimental and computational approaches, as they interact directly with cells (Rocha, Edroaldo, 2014; Islam, 2024).

Drug delivery, catalysis, diagnostics, and therapies have been extensively explored using nanoparticles composed of metals, polymers, and lipids. In the field of gene delivery, nanoparticles have gained interest due to their small size, which allows for cellular penetration, their large surface area for molecular cargo loading, and their ability to protect the transported molecules from external threats. Therefore, cellular reprogramming using nanoparticle-based gene or protein carriers has been employed to achieve sustained gene expression of reprogramming factors, improving reprogramming efficiency (Petros, 2010; Kuznetsova, 2024).

Non-viral delivery techniques, such as electroporation, micro/nanoparticles, nucleic acids, and modifications in substrate topography and stiffness, have provided valuable insights into cellular reprogramming (Joseph, 2018; Li, P. L., 2024). Topography, substrate stiffness, and mechanical stretching have been used to modify microenvironments and investigate their effects on cell proliferation and differentiation. These strategies aim to either enhance existing viral methods or reduce risks by employing non-viral mechanisms, which have the potential to increase reprogramming efficiency (Joseph, 2018; Wong, 2017).

For example, mesoporous silica nanoparticles (MSNs) have adjustable charge properties, reduced cytotoxicity, and high transport efficiency. MSNs possess a porous internal structure with a large surface area, allowing them to carry significant molecular cargo within the nanoparticle. These nanoparticles have proven to be useful in rapidly converting mouse iPSCs into definitive lineage cells (Mamaeva, 2013; Godakhindi, 2024).

Magnetic nanoparticles have been used for nanofection of iPS genes, a process that requires a magnetic field to induce transfection. For over a decade, this method, known as "magnetofection", has been employed to deliver non-viral genes with high efficiency (Scherer, 2002; Sendera, 2024).

Additionally, core-shell magnetic nanoparticles coupled with gene-targeting molecules and gene repression agents have been used to enhance neural stem cell differentiation into functional neurons by repressing gene expression in stem cells. Although these studies were not specifically designed to generate iPSCs or induce stem cell differentiation, they provide

valuable insights into how different nanomaterials influence cellular behavior, potentially accelerating the development of stem cell reprogramming and regenerative medicine technologies (Patel et al., 2015).

Solid lipid nanoparticles (SLNs) are formed by dispersing high-melting-point lipids, such as cetyl palmitate, in an aqueous medium. According to studies, one of the main advantages of SLNs is that they do not require organic solvents during production (Pardeike, J., 2009; Mallén, 2024).

SLNs can be administered via various routes, but they are commonly used in dermatological medications and cosmetics due to several advantages, including excellent biocompatibility, as they are composed of low--toxicity lipids, and efficient transdermal drug delivery due to their small size, which allows for high drug load capacity. However, one drawback is their high water content, which can affect product stability. Despite this, SLNs are widely employed in both national and international pharmaceutical markets (Pardeike, 2009; A. Subodh, 2024).

Polymeric nanoparticles provide a highly biocompatible, non-toxic, and biodegradable alternative. Various natural polymers, such as gelatin, alginate, and albumin, are used to create these nanoparticles. However, challenges such as reproducibility issues remain (Schaffazick, 2003; Fang, 2020). Among natural polymers, albumin possesses unique characteristics that make it an excellent drug delivery vehicle, particularly in oncology (Hawkins, 2008; Asrorov, 2024).

Liposomes are vesicular structures composed of one or more concentric lipid bilayers enclosing aqueous compartments. These systems exhibit high biocompatibility and biodegradability, particularly when composed of natural lipids. Their size, lipid composition, aqueous phase content, and surface properties can be easily modified to meet pharmaceutical and biomedical requirements. These characteristics make liposomal systems highly versatile, attracting significant research interest since the 1970s and leading to the introduction of various liposomal products into the market (Santos, 2002; Gatto, 2024).

Micelles are amphiphilic structures that have been extensively studied as drug delivery systems for topical, oral, and intravenous administration. Several micelle-based carrier systems have been reported in the literature, including mixed micelles, nanoparticulate micelles, and polymeric micelles. Mixed micelle systems consist of phospholipids and surfactants, commonly bile acids, arranged around a hydrophobic core containing a molecularly dispersed drug (Torchilin, Pharm. Res., 2007; Maboudi, 2024). Micellar nanoparticles, another micelle-based drug delivery system, were developed and patented by Novavax in 2003, leading to the commercial product Estrasorb®, a topical estradiol formulation. This system is composed of a multifaceted nanoemulsion formed by mixing a solvent, stabilizer, oil, and water under high pressure. The final formulation contains various micro/nanostructural components, such as free drug crystals, drug-associated oil droplets, micelles, or solubilized drug phases (Lee, 2010; Maboudi, 2024; Torres, 2024). Polymeric micelles are self-assembled amphiphilic systems composed of hydrophilic and hydrophobic polymer blocks. These systems form a core-shell structure, where a lipophilic drug is encapsulated within the hydrophobic core and interacts with the micellar hydrophobic domains, or is covalently bound to the polymers (Torchilin, Pharm. Res., 2007; Jin, 2024).

New pharmaceutical formulations have been developed using drug nanocrystals, a nanotechnology approach that significantly enhances oral drug bioavailability by reducing the time required to reach peak plasma concentration (Duran, 2010; Ding, 2024).

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CONCLUSION

In recent decades, cellular reprogramming has made substantial advancements in the field of gene therapy, including the development of increasingly sophisticated delivery systems with greater distribution potential and stability. Progress in genomics has provided a deeper understanding of the molecular foundations of genes and has identified various mechanisms and pathways that can serve as targets for gene therapy.

As gene therapy becomes more closely linked to cellular reprogramming, it will lead to continuous advancements and ongoing efforts to improve this technique and expand its applications. Although nanoparticle-based reprogramming is not yet as widely used as other therapeutic and clinical approaches, it is expected to evolve further with the advancement of nanoparticle synthesis techniques and a better understanding of their role in reprogramming.

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