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Review Article

Nanoscaffolds for Guided Cardiac Repair: The New Therapeutic Challenge of Regenerative Medicine

Letizia Ventrelli,[1,2] Leonardo Ricotti,[2] Arianna Menciassi,[2] Barbara Mazzolai,[1] and Virgilio Mattoli[1]

[1] *Center for Micro-BioRobotics @SSSA, Istituto Italiano di Tecnologia, Viale Rinaldo Piaggio 34, 56025 Pontedera, Italy*
[2] *The BioRobotics Institute, Scuola Superiore Sant'Anna, Polo Sant'Anna Valdera, Viale Rinaldo Piaggio 34, 56025 Pontedera, Italy*

Correspondence should be addressed to Letizia Ventrelli; l.ventrelli@sssup.it

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Cardiovascular diseases represent the leading cause of death and disability in the world. At the end-stage of heart failure, heart transplantation remains the ultimate option. Therefore, due to the numerous drawbacks associated with this procedure, new alternative strategies to repair the wounded heart are required. Cell therapy is a potential option to regenerate functional myocardial tissue. The characteristics of the ideal cardiac cell therapy include the use of the proper cell type and delivery methods as well as the choice of a suitable biomaterial acting as a cellular vehicle. Since traditional delivery methods are characterized by several counter backs, among which low cell survival, new engineered micro- and nanostructured materials are today extensively studied to provide a good cardiac therapy. In this review, we report the most recent achievements in the field of cell therapy for myocardial infarction treatment and heart regeneration, focusing on the most commonly used cell sources, the traditional approaches used to deliver cells at the damaged site, and a series of novel technologies based on recent advancements of bioengineering, highlighting the tremendous potential that nanoscaffolds have in this framework.

1. Introduction

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) represent the leading causes of death and disability in the world. Ischemic heart diseases such as myocardial infarction (MI), in particular, represent widely spread pathologies, producing significant morbidity [1]. MI is characterized by a decrease of blood supply to the cardiac tissue with a consequent death of cardiomyocytes and loss of contractile function. As a further consequence, the resulting nonfunctional tissue, which is still subjected to mechanical loads, generates abnormal stresses at the infarct and peri-infarct zone, with an expansion of the left ventricle [2]. The mechanical and biological stresses imply continuous changes at the structural, mechanical, and molecular levels, known as postinfarct left ventricle remodeling [3] (Figure 1). This process is highly dynamic and time dependent, comprising (i) an acute inflammatory phase, evolving to (ii) a granulation stage and then to (iii) chronic fibrosis. This degeneration progress often culminates in heart failure and death.

At the end-stage of heart failure, heart transplantation remains the ultimate option. However, the procedure of replacing the failed heart with a healthy one raises several limitations such as lack of organ donors, immune rejection, and many other complications. Because of these restrictions, researchers are still looking for new alternative strategies to repair the wounded heart and permanently restore its function. Among all approaches, cell therapy is a potential option to regenerate functional myocardial tissue. Stem or nonstem cell-based procedures are of great interest at present, and they hold great promises for a significant recovery of cardiac function. As known, the therapeutic effect of exogenous stem cells is due to four main general mechanisms: (i) differentiation of the administered cells into the cellular constituents of the regenerating myocardium; (ii) release of factors capable of paracrine signaling (a form of cell signaling in which the targeted cell is close to the signal-releasing one) from the administered cells; (iii) fusion of the administered cells with the existing constituents of the target organ; (iv)

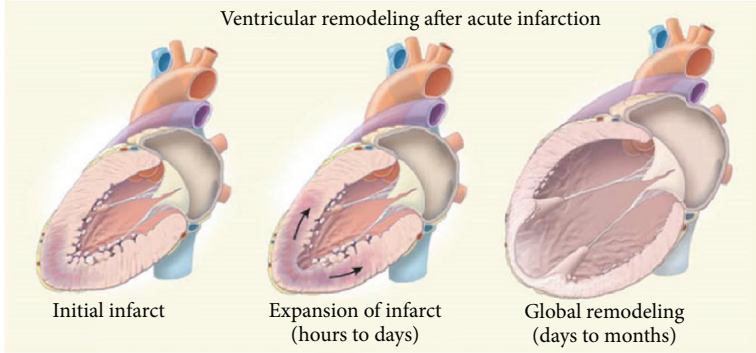

Figure 1: Ventricular dilation associated with progressive heart failure. After the initial insult, infarct expansion and ventricular wall thinning contribute to further ventricular remodeling, ultimately causing increased intraventricular pressure and decreased cardiac output. Reproduced with permission of Elsevier.

restoration of endogenous stem cell niches in the target organ, stimulated by exogenous cell delivery [4]. However, nonstem cells have been also used for therapeutic purposes, such as autologous or heterologous myoblasts.

The characteristics of the ideal "cardiac-therapeutic" cell type include quantitative and temporal availability, safety of administration, effectiveness of its engraftment, differentiation ability, and (most importantly) cardiac repair capability [5]. However, cell activity is not the only concern within this picture; delivery methods and properly engineered biomaterials actually represent other two key aspects to consider. As discussed in Section 3, all traditional delivery methods lead towards low cell survival and/or limited therapeutic efficacy [6]. Conversely, engineered micro- and nanostructured materials are widely studied at present to permit a suitable cell differentiation and integration with the host tissue as well as to release active compounds or to directly or indirectly perform physical therapy. Cells in general and stem cells in particular are strongly affected by extracellular stimuli, such as soluble and adhesive factors, which bind to cell-surface receptors. However, mechanical properties of the extracellular matrix, especially rigidity, also play a key role in cell signaling, proliferation, differentiation, and migration [7, 8]. Similarly, topography is able to trigger specific behaviors and/or to inhibit certain pathways [9]. The development of properly engineered substrates mimicking natural stem cell environment is therefore a crucial research field. In addition, new active materials and highly technological integrated devices are emerging, with the aim of providing injured heart with locally delivered drugs and indirect or direct physical stimulation [10–13]. Despite such synergistic joint efforts of engineers, biologists, and material scientists, a number of scientific and technological issues remain to be addressed and solved before fully exploiting the potential of cell-based regenerative therapies for heart-related pathologies. This will be probably possible in a near future by means of even more pronounced interdisciplinary research efforts [14].

This review aims to report the most recent achievements in the field of cell therapy for MI treatment and heart regeneration and to highlight the tremendous potential that nanoscaffolds have within this challenge. Firstly, the most common cell sources used in the last decades for cardiac repair will be described showing also the most recent clinical studies on humans. Then, the traditional approaches used to deliver cells at the damaged site will be reported, discussing their safety and their efficacy in inducing cardiac remodeling and healing. A series of novel technologies, based on recent advancements of bioengineering will be then exposed, including patches, fragments, active scaffolds, composite biomaterials, and so forth. Finally, an original strategy for cardiac repair based on magnetic nanosheets will be highlighted and discussed, before drawing conclusions about the promises of these new systems in the therapeutic challenge of heart regeneration.

2. Sources of Cells for Cardiac Remodeling

Different cell sources and types determine different therapeutic outcomes. Cell-based treatment of cardiac pathologies can be carried out by using either stem or nonstem cell types. Concerning stem cells, their level of commitment is strongly related to the therapeutic potential but also to possible drawbacks, such as teratoma formation [15]. Based on the differentiating potential, stem cells can be classified into three categories. Pluripotent stem cells, such as induced pluripotent stem cells (iPSCs) and human embryonic stem cells (hESCs), show the greater healing potential thanks to their ability to differentiate into cells of all three embryonal layers (ectodermal, endodermal, and mesodermal). Multipotent stem cells, such as mesenchymal stem cells (MSCs), can generate many cell types (e.g., muscle cells, hepatocytes, blood cells, etc.) within a specific organ. Finally, oligopotent stem cells (such as myeloid or lymphoid precursors) can generate only few cell types (e.g., monocytes, macrophages, etc.), and they are generally not taken into consideration for cardiac repair.

Consistent differences can be also found between stem cells of the same type but deriving from different sources. For example, a comparative analysis between MSCs of different origin revealed that those deriving from umbilical cord blood show a limited isolation success rate and are not able to differentiate in adipose tissue; however, they can be cultured

for long periods and show a high proliferation capacity. On the contrary, MSCs from bone marrow show high isolation success rate and good differentiation capacity but very low proliferation ability. Finally, MSCs from adipose tissue show the highest colony frequency and good differentiation ability [16].

hESCs and iPSCs have not been used in clinical studies yet for the treatment of myocardial disease, due to a lack of knowledge about their tendency to form tumor tissues when used *in vivo* and to strong ethical issues related to the use of human embryos (concerning hESCs) and to epigenetic memory and other partly unknown phenomena related to the use of iPSCs. However, the therapeutic potential of these cells have been evidenced by many studies both *in vitro* [17–20] and *in vivo* [21, 22], which highlighted the possibility of efficiently differentiating pluripotent cells into cardiomyocytes and, in general, of triggering cardiac regeneration.

Adult MSCs showed good promises not for their capability of directly differentiating into cardiac-like tissue, but rather for their ability to induce "trophic effects" [23]. These effects include secretion of cytokines and growth factors, inhibition of fibrosis (and therefore prevention of scar formation) and apoptosis, angiogenesis enhancement, and stimulation of tissue-intrinsic reparative processes. Encouraging results were obtained by using adipose tissue-deriving MSCs in rat myocardial infarct models [24] and bone marrow-derived MSCs in pig with damaged myocardium [25]. Concerning MSC application to humans, the outcomes obtained in eighteen clinical studies in which bone marrow-derived cells (BMCs) were used for cardiac repair were reviewed and meta-analyzed in 2007 [26]. The results highlighted that BMC transplantation is associated with modest improvements in physiologic and anatomic parameters in patients with both acute myocardial infarction and chronic ischemic heart disease. However, research is still focused on the translation of MSCs to the clinics by focusing on specific heart defects or impairments [27].

Cardiac progenitor cells (CPCs) have been studied in postnatal hearts, and specific surface markers expressed by these cells have been identified. The most studied are the CPCs expressing the tyrosine kinase receptor c-KIT [28]. Endogenous c-KIT$^+$ cells (such as interstitial cells of Cajal, thymic epithelium cells, and mast cells) are therefore under study, in order to identify their regeneration potential. CPCs from adult myocardium have been already reported to give rise to cardiomyocytes *in vitro* and *in vivo* after transplantation and to enhance cardiac function after infarction [29]. The "stemness" of CPCs has recently been questioned, and it has been suggested that they are principally cardiac fibroblasts, while CPC-derived cardiomyocytes are contaminants derived from the original tissue [30].

Concerning other cells used for cardiac repair, the literature reports heterologous epicardium-derived cells [31], skeletal myoblasts derived from skeletal muscle satellite cells [32–34], fetal cardiomyocytes [35], fibroblasts, and smooth muscle cells [36, 37]. In all these cases, even if some beneficial effects were found to the infarcted heart (mainly due to paracrine effects), the overall regeneration outcomes were rather poor.

Another interesting possibility has been recently highlighted by Song and colleagues [38], who reprogrammed cardiac fibroblasts into myocardial cells by using cardiac transcription factors (GATA4, HAND2, MEF2C, and TBX5) in mice.

In general, many years of *in vitro* and *in vivo* experiments and clinical trials have permitted to draw some conclusions [39]. Cell therapy is overall safe, with the caveat of ventricular arrhythmias which still require careful scrutinization; the cell type needs to be tailored to the primary clinical indication, whereas the paracrine effects of bone marrow cells may be therapeutically efficacious for limitation of remodeling or relief of angina. Only cells endowed with a true cardiomyogenic differentiation potential are likely to affect regeneration of chronic scars; autologous cells are primarily limited by their variable and unpredictable functionality, thereby calling attention to banked, consistent, and readily available allogeneic cell products. Regardless of the cell type, a meaningful and sustained therapeutic benefit is unlikely to occur until cell transfer and survival techniques are improved to allow greater engraftment rates. Furthermore, trial end points probably need to be reassessed to focus on mechanistic issues or hard end points depending on whether new or already extensively used cells are investigated.

3. Traditional Approaches for the Delivery of Cells in the Injured Heart

In addition to the cell type to be transplanted, another key factor concerns the adoption of an efficient method for cell delivery. The main objectives are (i) to ensure a safe transplantation, (ii) to transplant a sufficient amount of cells into the cardiac region of interest, (iii) to obtain maximum retention of cells within the target area, and (iv) a sufficient local engraftment [40]. Although there is a number of available options to direct cells to the heart, in this section, we focus on three basic strategies, namely, systemic therapy, focused coronary infusion and direct myocardial injection.

3.1. Systemic Therapy. Systemic administration of cells can be achieved through both growth factors mobilization and peripheral venous injection [40].

In the growth factor treatment [41], myocardial regeneration via stem cells mobilization and migration from tissues to injured myocardium is favoured by using specific growth factors like stromal cell-derived factor-1 (SDF-1) and stem cell factor (SCF). In particular, when the natural cell processes are not sufficient for MI healing, these phenomena are artificially induced and accelerated by supra doses of such chemical agents. Askari et al. [42] investigated the expression of SDF-1 by the myocardium after MI, finding a significant upregulation immediately after infarction and a downregulation within 7 days; the results suggested that SDF-1 is sufficient to induce therapeutic stem-cell homing to MI. Finally, though some findings showed general enhancement of cardiac performance *per se*, the efficacy of the growth factor therapy is improved when the treatment is combined with standard cellular transplantation [43].

Compared with invasive delivery methods such as direct intramyocardial injection, intravenous (IV) injection of cells [44, 45] is the simplest and noninvasive delivery strategy to treat myocardial diseases in humans. Moreover, the administration of a large numbers of cells can be easily repeated. Peripheral infusion of stem cells (by the jugular vein, the femoral vein, the scalp vein, etc.) is also used in small animal (e.g., rats) and porcine models of MI showing limited myocardial infarct size, reduced remodeling, and improvement of cardiac function [46–48]. However, entrapment of cells to noncardiac organs such as the lungs [45], lowering the selectivity and efficiency of this approach, limits its applicability.

3.2. Intracoronary Infusion. Selective intracoronary infusion of cells in the proximity of ischemic myocardial areas allows the delivery of a higher cell concentration in comparison with systemic therapies. Successful experimental [49] and clinical [50] studies have recently showed that BMCs and MSCs delivered by the intracoronary route regenerated damaged myocardium in acute MI. Chen at al. [51] investigated the efficacy of intracoronary injection of BMCs in patient with acute MI, confirming significant improvement on cardiac function and on left ventricle remodeling. The infusion of cells to the injured myocardium carried out in these studies is technically simple to perform: cells are injected through the central lumen of an over-the-wire balloon catheter during transient balloon inflations in order to maximize the exposure time of cells with the microcirculation of the infarct-related vessel. Moreover, clinical trials also demonstrated the feasibility, safety, and efficacy of this method [52]. However, possible drawbacks may be the nonselective distribution of the injected cells and, depending on the delivered cell type, their ability to migrate from coronary vessels and to infiltrate into the infarcted site without causing obstruction.

3.3. Direct Intramyocardial Injection. Direct myocardial injection is suitable to deliver cells in patients with chronic myocardial diseases, such as chronic ischemia [53], or other advanced coronary artery diseases [54], but it could be also used to treat acute MI. Direct injection can be realized transepicardially, transendocardially, or via the coronary venous system [40].

Transepicardial cell injection can be performed during open heart surgery, allowing for a direct visualization of the myocardium. Due to its invasiveness, this technique is commonly used in animal studies, whereas its clinical application is limited to patients undergoing sternotomy for different cardiac surgery. Menasché et al. [55] transplanted autologous skeletal myoblasts in patients undergoing coronary artery bypass grafting operations via multiple epicardial echo-guided needle injections. Even if this first clinical trial failed and no improvements in regional or global LV function were found, the increased number of early postoperative arrhythmic events after cell injection and LV remodeling for high-dose injection laid the groundwork for future investigations.

Another possible strategy for direct cell injection is the less invasive transendocardial delivery via a percutaneous catheter-based approach [56]. To date, a multiplicity of catheter systems are available for transendocardial injection such as the Stiletto [57] or MyoStar [58] catheters. Basically, all these systems are made up of multicomponent catheters consisting of an injection needle for cell delivery and a support catheter to direct the needle to the desired site. Therefore, by passing the catheter retrogradely across the aortic valve, it is placed against the endocardial surface, thus allowing intramyocardial cell injection into the left ventricular (LV) wall. Moreover, some of the catheter systems described before integrate additional navigation capability to facilitate the selection of target areas. Perin et al. [59] performed an electromechanical mapping (EMM) of the endocardial surface by integrating the MyoStar catheter with the NOGA system; this study demonstrated the safety of intramyocardial injection of bone marrow-derived stem cells in patients with severe LV dysfunction and an improvement in both perfusion and myocardial contractility.

The last emerging technique for direct cell repopulation is transcoronary vein injection. In this approach, cells can be intramyocardially injected through the coronary veins by using a catheter placed inside the coronary vein itself. In particular, Thompson et al. [60] reported a study in which the coronary venous system of pigs was used as a road map for direct cell injection. For this purpose, a special catheter system incorporating an intravascular phased array ultrasound tip for guidance and a preshaped extendable nitinol needle for transvascular myocardial access were used. While they demonstrated the feasibility (e.g., the widespread intramyocardial access from the anterior interventricular coronary vein) and safety (e.g., no death, no ventricular arrhythmia, or other procedural complications) of percutaneous intramyocardial access, the efficiency of cell grafting or functional properties of the myocardium after the injection were not assessed. Nevertheless, the study carried out by Thompson is an important step regarding the treatment of myocardium via the coronary venous system.

In contrast to peripheral venous injection and intracoronary infusion, where the specificity of the delivery is very low, direct intramyocardial injection of cells represents a more attractive route. This is due to the specific regions of the myocardium that can be targeted (noncardiac entrapment of the cells is reduced) and, as a consequence, to a highest local tissue concentration that can be reached.

Finally, based on both the advantages and drawbacks of the delivery methods previously reported, it is possible to conclude that the choice of the more suitable strategy depends on several factors, such as the specific disease the patient is affected by and the type of cells to be transplanted. For this reason, and as a consequence of the fast and sophisticated technological improvements we are witnessing, further studies and more clinical trials should be performed in the near future.

4. New Approaches: Cell Sheet Engineering, Fragments, and Patches

This section aims to report tissue engineering technologies recently developed for the treatment of MI. The most

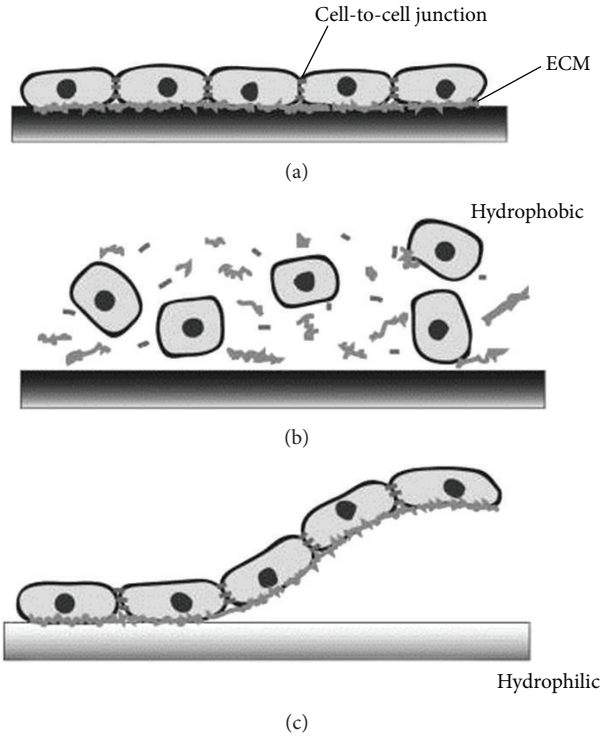

Figure 2: Cell sheet detachment from temperature-responsive culture surfaces. (a) ECM assures cell attachment to hydrophobic surfaces, and cell-to-cell junction proteins keep cells packed together; (b) enzymatic digestion causes ECM, and junctions are disrupted, and cells are released separately; (c) thermoresponsive culture surfaces allow the release of cell sheets by lowering temperature. Reproduced with permission of Wiley & Sons.

important features of these techniques are summarized in Table 1.

In the nineties, Okano's group developed an innovative tissue engineering method named "cell sheet technology" [61, 62]. Such technology is based on thermoresponsive culture dishes designed by using a temperature-responsive polymer, poly(N-isopropylacrylamide) (PNIPAAm), covalently immobilized onto common tissue culture polystyrene surfaces (TCPSs). By controlling the hydrophobicity of the dish through temperature, it is possible to switch on and off cell adhesion (Figure 2).

Through this method the seeded cells detach spontaneously and can be harvested in a noninvasive way as an intact sheet together with their deposited extracellular matrix (ECM). In comparison with conventional tissue engineering methodologies, cell sheet engineering shows several advantages. Firstly, there is the possibility to harvest cells as an entire sheet without using proteolytic enzymes such as trypsin, which causes the breaking of adhesive proteins and membrane receptors; furthermore, the presence of ECM on the sheet allows the sheet to be directly transplanted into host tissues without any mediators. Another important point is the elimination of biodegradable scaffolds, thus reducing the inflammatory response after implantation. Finally, the ability to harvest cell sheets as both single layers and multilayers (thus creating three-dimensional structures) paves the way to advanced regenerative therapies, not only for the treatment of MI. As regards myocardial reconstruction, cell sheets obtained from PNIPAAm-grafted TCPS have been used with different sources of cells [61, 63–66] and with cardiomyocyte sheets layered in different numbers (from two up to four). *In vitro* histological analyses showed an integration of the single sheets, resulting in a homogeneous, continuous, and cell-dense structure; moreover, thanks to the formation of gap junctions after the layering, electrically synchronized cell pulses were observed. *In vivo*, spontaneous beatings were macroscopically noticed, and typical heart-like structures were found out.

Sung's group proposed an alternative approach, relying on the same philosophy of cell sheet engineering [67–69]. A continuous cell sheet was harvested by using a thermoresponsive methylcellulose (MC) hydrogel coated on TCPS dishes; then, fragmented cell sheets were obtained by means of a stainless screen. Finally, the collected fragments were transplanted by injection through a needle (Figure 3).

In these studies, different cell types were used. Chen et al. [67] and Wang et al. [68] reported fragmented sheets of rat bone marrow MSCs, transplanted via intramyocardial injection directly into the periinfarct area using a needle. After sheet preparation, both studies confirmed the preservation of endogenous ECM; subsequent to injection, the MSC sheet fragments showed the maintenance of their activity. In comparison with traditional delivery methods, a higher number of MSCs were retained in the interested area, thus resulting in a higher heart recovery. In a more recent work, Yeh et al. [69] used human amniotic fluid stem cells (hAFSCs), known to have angiogenic capability and cardiomyogenic potential. After hAFSC isolation and expansion, cell sheet fragments were prepared and transplanted into the peri-ischemic area of a rat model. Again, the fragments preserved the endogenous ECM, thus leading to enhanced cell retention in the area of interest, and significant improvements in the cardiac function were also observed.

Cardiac patches represent another interesting approach for the treatment of heart pathologies [70–73]. Although heart patches can be developed in different ways, they are basically developed starting from both biological and synthetic scaffolds laden with cells. Figure 4 shows an example of cardiac patch.

Therefore, the two most important features being addressed during the development of cardiac patches are (i) the choice of biomaterials to be used as scaffolds (showing suitable mechanical properties, such as sufficiently strength to resist the movement of myocardial tissue, adjustable biodegradation times, and ability to both carry and preserve cells) and (ii) the choice of proper cell sources for myocardial repair. Based on these assumptions, several studies can be found in the literature. Piao et al. [70] used rat bone marrow-derived mononuclear cells (BMMNCs) seeded onto a poly-glycolide-*co*-caprolactone (PGCL) scaffold; its implantation into the epicardial surface of a rat MI model produced migration and differentiation of these cells towards cardiomyocyte-related phenotypes. Chen et al. [73] also fabricated a hybrid heart patch, demonstrating its capability

Table 1: Summary of recent tissue engineering technologies in cardiac failure.

Approach	Authors and year	Applications	Cell types	Materials	Delivery routes	Reference
Cell sheet engineering	Kikuchi and Okano, 2005 Matsuda et al., 2007 Shimizu et al., 2003 Masuda et al., 2008 Yang et al., 2007 Yang et al., 2006	Severe heart failure, myocardial tissue reconstruction	(i) Chick embryo cardiomyocytes (ii) Neonatal rat cardiomyocytes (iii) Skeletal myoblasts (iv) MSCs (v) Coculture of cardiomyocytes and endothelial cells	Temperature-responsive culture dishes: PNIPAAm grafted to TCPS	(i) Transplantation into dorsal subcutaneous tissues (ii) Direct transplantation into host damaged heart	[61–66]
Cell sheet fragments	Chen et al., 2007 Wang et al., 2008 Yeh et al., 2010	MI	(i) Rat bone marrow MSCs (ii) hAFSCs	Thermoresponsive MC hydrogel coated on TCPS dishes	Transplantation via direct intramyocardial injection	[67–69]
Tissue-bioengineered cardiac patches: scaffolds (biological or synthetic) laden with cell culture system	Piao et al., 2007 Wei et al., 2008 Huang et al., 2010 Chen et al., 2010	Heart failure associated with MI	(i) Rat BMMNCs (ii) Rat bone marrow MSCs (iii) Cardiomyocytes differentiated from hESCs (iv) Murine ESCs	(i) Porous acellular bovine pericardia (ii) PGCL (iii) PGS (iv) Thermoresponsive MC hydrogel system coated on TCPS dishes	Implantation and suture over different infarcted areas	[70–73]
(i) Cell sheets (ii) Bioengineered patches (iii) Hydrogels	Scudellari, 2009	Heart diseases	(i) Skeletal myoblasts (ii) BMCs (iii) MSCs (iv) Cardiac stem cells (v) ESCs (vi) iPS	(i) Biomaterials (ii) Biological constructs	(i) Direct injection into the heart muscle (ii) Percutaneous injection catheter-based delivery	[74]

MSCs: mesenchymal stem cells; PNIPAAm: poly(N-isopropylacrylamide); TCPS: tissue culture polystyrene; MI: myocardial infarction; hAFSCSs: human amniotic fluid stem cells; MC: methylcellulose; BMMNCs: bone marrow-derived mononuclear cells; hESCs: human embryonic stem cells; ESCs: embryonic stem cells; PGCL: poly-glycolide-*co*-caprolactone; PGS: poly(glycerol sebacate); BMCs: bone marrow cells; iPS: induced pluripotent stem cells.

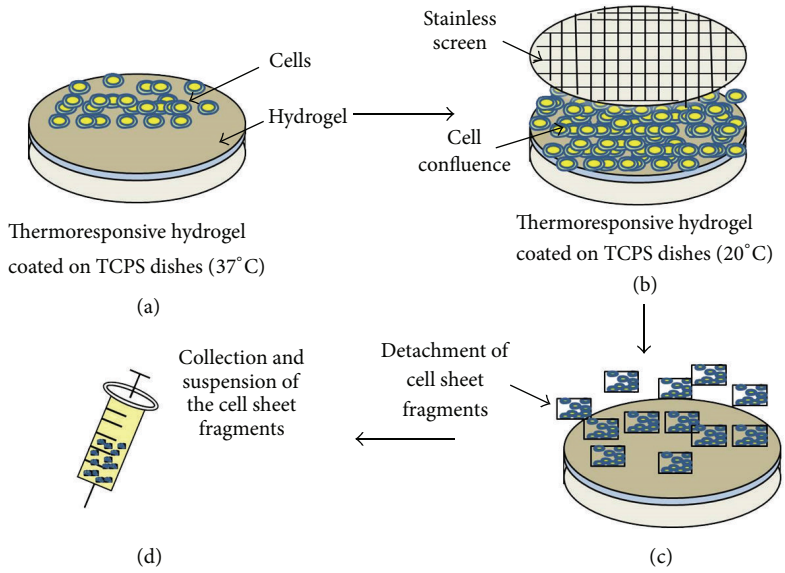

Figure 3: Representation of cell sheet fragments preparation and suspension. Cells cultured on a thermoresponsive hydrogel (a) are allowed to reach confluence and then fragmented by means of a stainless screen (b). Fragments are detached (c), suspended, and collected in a syringe (d) to be injected in the host organ.

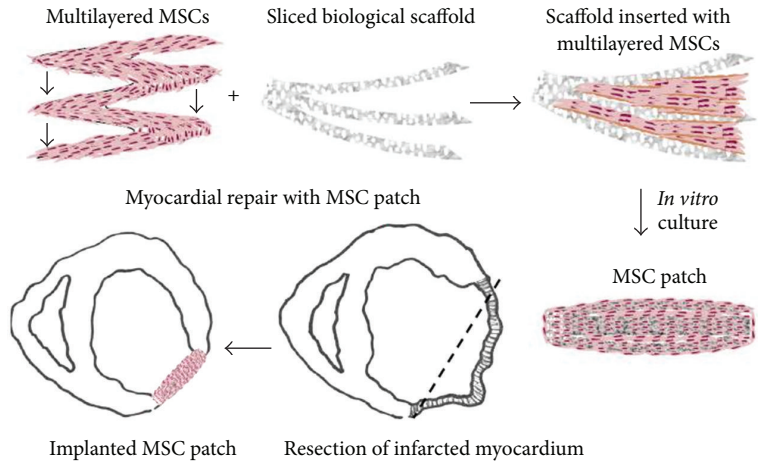

Figure 4: Scheme of preparation of a MSC-based therapeutic cardiac patch. Reproduced with permission of Elsevier.

to sustain cell viability and attachment as well as active cell beating for long periods; moreover, *in vivo* tests showed that the implanted patch remained undamaged over two weeks without affecting ventricular function. In this case, the cardiac patch was fabricated using again a synthetic scaffold made of poly(glycerol sebacate) (PGS), seeded with different cell types. In addition to synthetic scaffolds, biological materials can also be used to fabricate cardiac patches. Wei et al. [71] and Huang et al. [72] used sliced porous biological scaffolds (e.g., acellular bovine pericardia) and thermoresponsive MC hydrogels coated on TCPS dishes and seeded with rat bone marrow MSCs and murine ESCs, respectively. In both studies, *in vitro* tests showed high cell viability and adhesion, whereas a good integration into the host and an improvement of heart functions were observed during *in vivo* test.

Since all these bioengineering-based components have to be implanted into the human body, their future clinical applications will firstly require an eligible candidate and proven safety of both cell sources and biomaterials. A good outline of several newly developed tissue engineering techniques for the treatment of heart diseases is reported in [74].

A deep inside analysis of both traditional methods of cell delivery and the new bioengineering-based ones highlight that, despite the direct cell injection should be an inefficient route of delivery because of a high loss or death (more than 90% [75]) of cells, the combination of these approaches could prevent cell loss augmenting the cell transfer efficiency and giving a site-directed repair.

Finally, special attention has to be paid to biomaterials because, if the cell sheet engineering avoids the use of

Table 2: Advantages and disadvantages of novel tissue engineering technologies for the treatment of heart diseases.

Approach	Advantages	Disadvantages
Cell sheet engineering	(i) Elimination of the use of biodegradable scaffolds, increased cell-to-cell connections, and reduced inflammatory responses (ii) Noninvasive cells harvesting as intact monolayer sheets: cultured cells detach spontaneously by lowering temperature, thus avoiding the use of proteolytic enzymes (iii) Harvested cell sheets can be easily transferred and directly attached to the host tissue (iv) Ability to layer the harvested cell sheets: rapid cell-to-cell connections between the layers	(i) Transplantation of cell sheets alone cannot provide the proper mechanical strength necessary for replacing the infarcted myocardium (ii) Need to rely on open surgery
Cell sheet fragments	(i) No use of proteolytic enzymes when harvesting cells (ii) Injectable without open surgical methods (iii) Good ability on cell attachment and proliferation when transferred to other surfaces (iv) High cell retention in the infarcted myocardium	(i) Lack of a controlled delivery inside vessels (ii) Risk of thrombosis inside vessels
Bioengineered cardiac patches	(i) Integration of the advantages of tissue-engineered scaffolds and cell sheets (ii) Ability to provide the required mechanical strength to support seeded cell sheet systems (iii) Prevention of cell loss to augment cell transfer efficiency, thus allowing a site-directed repair	(i) Immunogenicity caused by residual degraded scaffolds (ii) Invasive open chest surgery (iii) Materials should be optimized according to the cell source

biodegradable scaffolds reducing inflammatory responses, they are used to develop cardiac patches involving the choice of the more suitable material up to the cell source. Table 2 aims at resuming an exhaustive overview of the main advantages and disadvantages of the above described novel tissue engineering technologies.

5. Advanced Biomaterials for Heart-Related Applications

As mentioned, the achievement of effective MI treatments depends not only on the choice of the most suitable cell source but also on the employed biomaterials. Since a biomaterial, especially if used for clinical trials, interacts with biological structures, the following main requirements are demanded [76]: (i) biocompatibility with human body in general and cardiac tissues in particular; (ii) safe biodegradability; (iii) specific mechanical properties resembling those of the native heart, such as strength and flexibility; and (iv) contraction capability. An exhaustive list of biomaterials used in the last decade for MI treatment is reported in Table 3, while Figure 5 shows, for some of them, their chemical structure, their degradation times, and representative images of 2D or 3D scaffolds.

The source of the material (i.e., natural, synthetic, or composite-derived) marks several differences between its properties and possible applications [76–81]. Furthermore, its delivery strategies should be also taken into account (i.e., injection, 3-D scaffolds, or patches) [77, 79, 81].

Concerning naturally derived materials, such as alginate, chitosan, collagen, and fibrin are the most used ones. Alginate is an anionic polysaccharide found in the cell walls of brown seaweed and, thanks to its biocompatibility and gelation with divalent cations (such as Ca^{++}) of the myocardial tissue, it shows good potential for MI treatment. Leor et al. [82] prepared an alginate-calcium solution that was intracoronary injected into a swine model of MI: the solution diffused into the infarcted zone and replaced the damaged ECM, thus preventing LV dilation. Chitosan is another linear (cationic) polysaccharide. Because of its biocompatibility and biodegradability, it is often used in biomedical applications ranging from drug delivery to tissue engineering. Wang et al. [83] intramyocardially injected a temperature-responsive chitosan hydrogel provided with specific growth factors into rat infarction models, obtaining significant improvements in cardiac functions. Fibrin glue, a biomaterial made up of fibrinogen and thrombin, can be also used for controlled release of growth factors. Nie et al. [84] demonstrated an enhancement in myocardial perfusion and cardiac functions in a canine infarct model by means of fibrin glue incorporating basic fibroblast growth factors. As described previously, all these materials can be either injected or used as delivery vehicles. An in-depth description of biological materials (concerning structure and mechanical properties) can be found in [85].

Synthetic materials represent another category of bioscaffolds used for heart regeneration. Widely used biodegradable and biocompatible polymers are poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymers, for example, poly(lactic-*co*-glycolic acid) (PLGA) [76, 77, 80]. Caspi et al. [86] reported the achievement of an engineered human cardiac tissue showing good contracting ability, thus demonstrating the capability of developing a highly vascularized tissue with specific cardiac structures and functions. The 3D biodegradable polymeric scaffold used in this study was half made of PLGA and the other half of another widely used polymer, poly(L-lactic acid) (PLLA). This substrate was subsequently seeded with different cell culture combinations,

Table 3: Biomaterials for MI treatment reported in literature, in the last decade.

Biomaterial	Delivery strategies	Reference
Naturally derived materials		
Alginate	Injectable	[80]
	3D scaffold	[76–79]
Chitosan	Injectable	[77]
	Patches	[78, 79]
Collagen	Injectable	[80]
	3D scaffold	[76]
	Patches	[77, 78]
Extracellular matrix	Injectable	[76]
	Patches	[79]
Fibrin and fibrin glue	Injectable	[76]
	3D scaffold	[77–79]
Gelatin	Injectable	[76, 77]
Silk fibroin	Patches	[77]
Synthetic materials		
PEG	Injectable	[76]
	Films	[78]
PLA	3D scaffold	[76, 77, 80]
PGA	3D scaffold	[76, 77, 80]
PLGA	3D scaffold	[76, 77, 80]
PU	3D sheet	[76, 77]
PEU	3D scaffold	[77]
PEUU	3D scaffold	[76]
	Patches	[77]
TMC	Films	[76]
PTMC	Films	[77]
DLLA	Films	[76]
PNIPAAm	Cell sheet	[76]
	Injectable	[78]
PGCL	Patches	[76]
PGS	3D scaffold	[77]
PCL	Patches	[77]
Self-assembling peptides	Injectable	[78, 79]
Other synthetic hydrogels	Injectable	[79]
Composites materials		
ε-Caprolactone-*co*-L-lactide reinforced with PCLA, gelatin or PGA	Patches	[80]
PEUU with type I collagen	3D scaffold	[76]
Poly(caprolactone) with type I collagen	3D scaffold	[76]
Decellularized materials		
Urinary bladder matrix, SIS	Injectable	[78]
Porcine ventricular and pericardial tissue	Patches	[78]

MTE: myocardial tissue engineering; 3D: three dimensional; PEG: poly (ethylene glycol); PLA: poly(lactic acid); PGA: poly(glycolic acid); PLGA: poly(lactic-*co*-glycolic acid); PU: polyurethane; PEU: polyester urethane; PEUU: Poly(ester urethane) urea; TMC: 1,3-trimethylene carbonate; PTMC: poly(1,3-trimethylene carbonate); DLLA: D,L-lactide; PNIPAAm: poly(N-isopropylacrylamide); PGCL: poly-*co*-caprolactone; PGS: poly(glycerol sebacate); PCL: poly(ε-caprolactone); PCLA: poly(L-lactide); SIS: small intestine submucosa.

each one based on the use of hESCs; by adding endothelial cells (ECs) and embryonic fibroblasts (EmFs), vascularization was finally encouraged. Such triculture system (composed of fibroblasts, endothelial cells, and cardiomyocytes) was also reported by Iyer and Radisic [76, 87], who used poly(ethylene glycol) (PEG) scaffolds as substrates for cell seeding.

Poly(ε-caprolactone) (PCL) [76, 77] is a biodegradable polyester which is degraded, in physiological conditions, by hydrolysis of its ester linkages; for this reason, it has been approved by the Food and Drug Administration (FDA) and, today, is widely used in several biomedical applications. PCL is often copolymerized with glycolide, resulting in poly(glycolide-*co*-caprolactone) (PGCL). Piao et al. [70, 76] fabricated cardiac patches made of PGCL and seeded them with BMMNCs; therefore, implants in rat MI models were performed, and a reduced LV remodeling and systolic dysfunction were achieved.

A recently developed biodegradable material for soft tissue engineering purposes is a special elastomer, named poly(glycerol sebacate) (PGS) [73, 77]. Thanks to its mechanical properties, comparable to those of the heart tissue, it has been used as cardiac patch by Chen et al. as previously mentioned [73]. Finally, another interesting synthetic material is poly(N-isopropylacrylamide) (PNIPAAm), a temperature-sensitive polymer that switches its surface properties from hydrophobic to hydrophilic below physiological temperature. As already mentioned, this property has been used for cell sheet engineering approaches.

The combination of natural and synthetic materials allows the preparation of composite scaffolds. Some examples are represented by poly(ester urethane) urea (PEUU) blended with type I collagen [76], and ε-caprolactone-*co*-L-lactide reinforced with poly(L-lactide) (PCLA), gelatin, or PGA [80] (see Table 3).

Finally, decellularized materials (tissues and organs) have been also used as bioscaffolds for tissue engineering and regenerative medicine applications [71, 72, 78, 88]. Decellularization consists of the removal (by means of physical, chemical or enzymatic methods) of cells from a tissue or an organ, maintaining only its ECM. Even if the native source tissue/organ and the decellularization methods affect the structure of the ECM scaffold and therefore its response before and after the implantation in the body, this could represent a good way to provide cells with the best environment in which they can grow, proliferate, migrate, and differentiate. Furthermore, decellularization is helpful because the removal of antigens could avoid inflammations and, consequently, a transplant rejection. Singelyn and Christman [78] reported some examples of decellularized materials used for cardiac applications such as decellularized small intestine submucosa (SIS), intact rat and porcine hearts, and decellularized ventricular and pericardial ECM. In particular, Christman's group [78, 89] carried out *in vitro* and *in vivo* studies on an injectable decellularized myocardial matrix, made of porcine ventricular tissue, in order to characterize its composition and structure. The experiments showed the capability of myocardial matrix to gel both *in vitro* and *in vivo* upon epicardial injection, to self-assemble in nanofibrous scaffolds, and to promote both *in vitro* and *in vivo* migration of endothelial and smooth muscle cells within the structure.

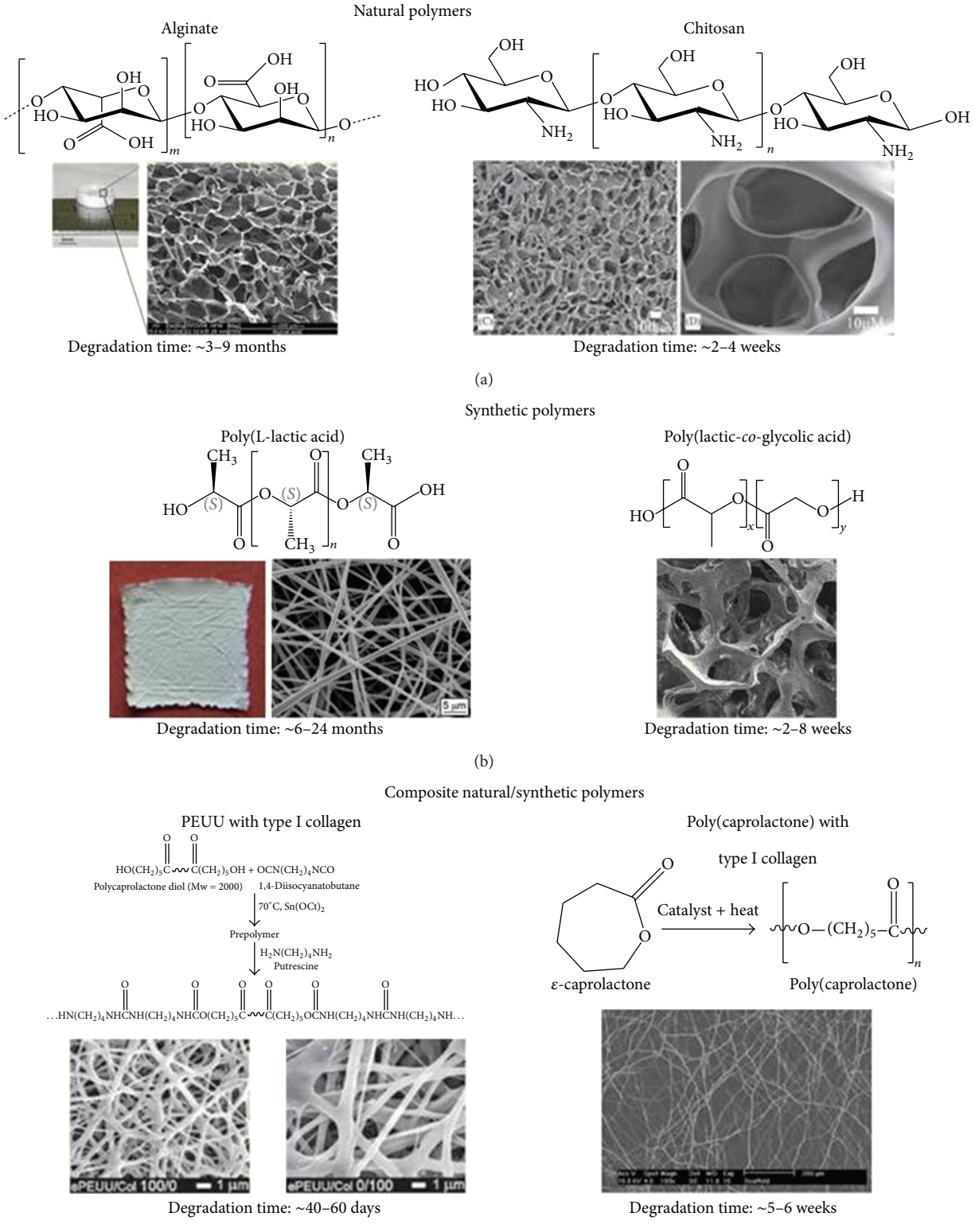

Figure 5: Biomaterials widely used for MI treatment. For each of them, the chemical structure (top), representative images of the scaffold (middle) and the degradation time (bottom) are reported. Reproduced with permission of Elsevier and Wiley & Sons.

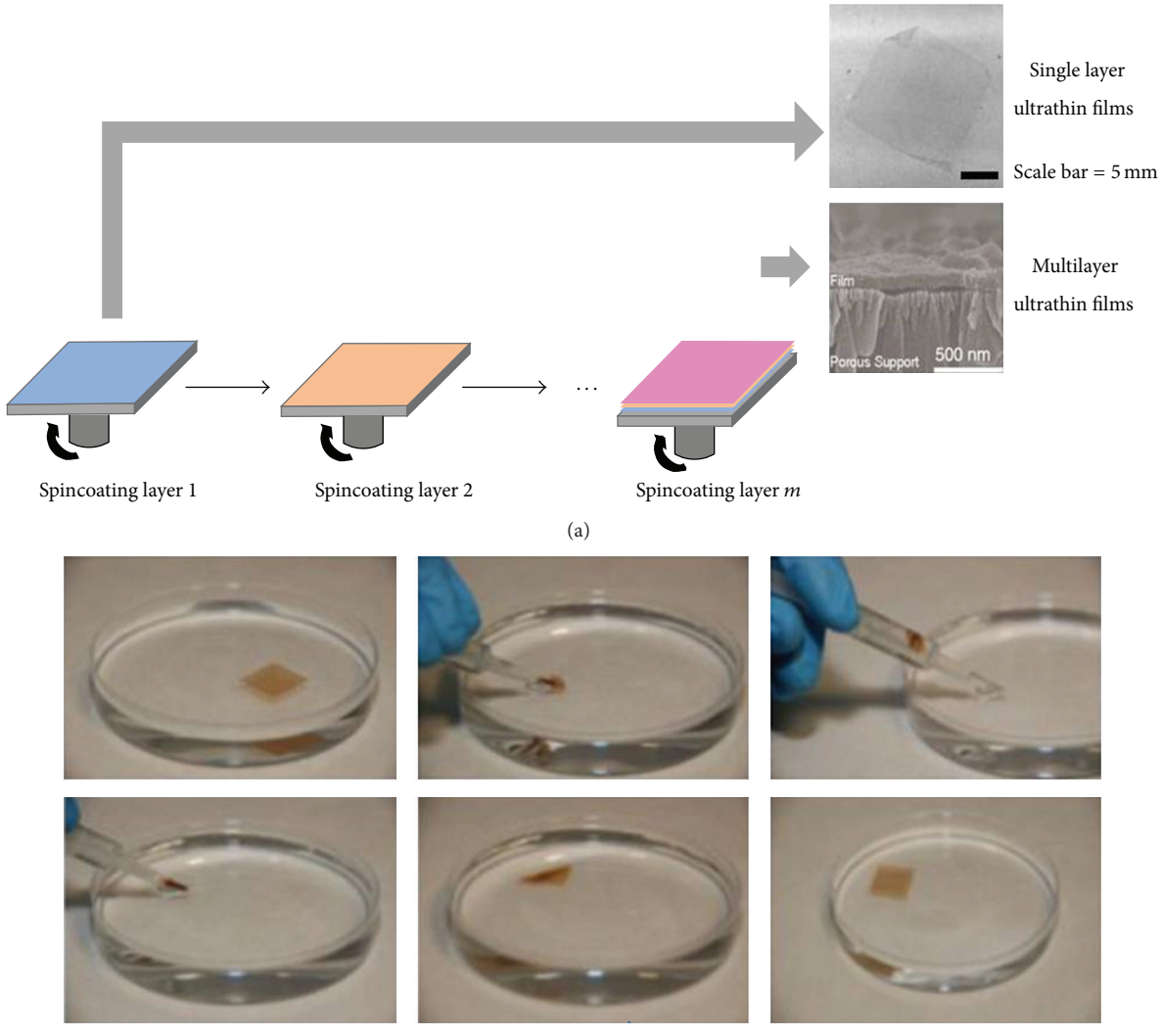

FIGURE 6: Polymeric nanofilms. (a) Scheme of single and multilayer nanofilm fabrication process; (b) magnetic nanofilms: injection (top) and ejection (bottom) sequence. Reproduced with permission of Elsevier, Springer, and Wiley & Sons.

6. Cells on Magnetic Nanosheets and Their Guidance

In addition to the already described technologies, an original approach based on polymeric nanofilms recently emerged, and it holds great promises for the treatment of heart diseases.

Nanofilms are polymeric films showing a relatively large area (~100 cm^2) and a nanometric thickness (10–100 nm), which leads to define them as "quasi 2D structures". Their flexibility, associated with robustness and high biocompatibility as well as the possibility to use numerous polymers to fabricate them, opens the way to several applications in the biomedical field, ranging from biochemical or physical sensors, drug delivery systems, or substrates for tissue engineering [90, 91]. Concerning their fabrication, polymeric nanofilms can be prepared both as single and multilayer films by means of a simple method firstly proposed by Takeoka's group, namely, spin coated assisted deposition [90]. In Figure 6(a), a simple scheme of the spin coating process is shown. This technique is used as a single or a multistep process, according to the required number of layers [92–94]. Two different approaches are exploited in order to produce ultrathin nanosheets [95, 96]. The former uses a water-soluble polymer as a sacrificial layer, which is directly spun onto a substrate via spin coating; subsequently, another layer of the desired polymer is deposited on top. In the latter, a polymeric layer is firstly deposed onto the substrate, followed by the spinning of the polymeric supporting layer.

Since one of the key purposes of ultrathin films concerns their use as drug or cell carriers (as discussed below), the handling, control, and positioning of nanofilms inside the human body represent crucial requirements. A possibility that recently emerged consists of the development of magnetic nanofilms, specifically nanosheets containing magnetic elements. This feature allows an external and noninvasive approach, based on the use of an outer magnetic field, which can be adopted for an accurate displacement of the film within the body. A recent study performed by Mattoli's group

Table 4: Novel approach based on ultrathin nanofilms.

Authors	Technology	Methods	Cell types	Materials	Possible applications	Reference
Pensabene et al., 2009 Mattoli et al., 2009	Polymer nanosheets bearing magnetic nanoparticles	Spin coating method		(i) PVA (ii) PLLA (iii) NPs	Biomedical field as free-standing carriers or as plasters	[95, 96]
Ricotti et al., 2010	Polymer nanosheets bearing cells	Spin coating method	C2C12 mouse myoblast cell line	(i) PVA (ii) PLA	(i) Regenerative medicine (ii) Muscle tissue engineering (iii) Drug delivery	[101]
Fujie et al., 2010	Polymer nanosheets bearing cells	Layer-by-layer assembly method	NIH-3T3 mouse fibroblast cell line	(i) COL (ii) HA	(i) Regenerative medicine (ii) Cell biology	[97]
Fujie et al., 2011	Polymer nanosheets bearing cells	Spin coating method	H9c2 embryonic myocardium rat cell line	(i) PVA (ii) PLLA	Building of specific cell culture environments	[99]

PVA: poly(vinyl alcohol); PLLA: poly(L-lactic acid); NPs: iron oxide nanoparticles; PLA: poly(lactic acid); COL: collagen; HA: hyaluronic acid.

[96] was based on the fabrication of nanofilms functionalized with paramagnetic nanoparticles (NPs). The magnetic behavior of the nanosheet, floating in water, was evaluated by using an external permanent magnet (field intensity Br = 350 mT): results showed a fine film controllability and highlighted that velocities were strictly correlated to the concentration of the loaded NPs. A practical application of magnetic nanofilms was described by Pensabene et al. [95]. In this work, magnetic nanofilms were developed and evaluated as both drug carriers and nanoplasters. Magnetic nanosheet manipulation was tested by using a micropipette (Figure 6(b)); adhesion to the gastric mucosa was also assessed. Furthermore, the control and positioning of the films in a liquid environment (water) were finally addressed, showing their ability to reach and attach on the tissue. These outcomes allowed to conclude that the use of magnetic nanofilms constitutes a feasible strategy for bringing and releasing in a controlled way drugs or cells in different areas of the human body. Of course, further studies will be required in order to assess both the proper concentration of magnetic components and the appropriate values of external magnetic fields.

As previously mentioned, free-standing polymeric ultrathin films can be also used as novel cell culture systems and cell carriers, in order to be applied to several biomedical applications (e.g., cell biology, tissue engineering and regenerative medicine applications, and included MI therapy). The most recent studies focusing on the adhesion and proliferation properties of cells seeded onto single layer or multilayer nanofilms are summarized in Table 4.

In [97], Fujie et al. reported the fabrication of two types (i.e., fibril and nonfibril) of free-standing ECM-like multilayer nanosheets made of biopolymers such as type I collagen (COL) and hyaluronic acid (HA). Both layer-by-layer (LbL) assembly method [98] and supporting technique [95, 96] were used. Subsequently, the morphological and mechanical features of the ECM-like nanosheets as well as their mechanical effects on the adhesive properties of NIH-3T3 mouse fibroblasts seeded on it were studied. Results showed that the elongation of cells increased on the fibril-based nanofilms, whereas it decreased on the nonfibril-based ones. This study demonstrated that cell adhesion properties were tuned by simply varying the structural ECM components of the nanosheets (e.g., the content of HA and COL fibrils). The mechanical effects of freely suspended polymeric nanofilms on cell adhesion properties have been also assessed by studying how nanosheet stiffness affects biological responses. Fujie et al. [99] synthesized ultrathin films, composed of PLLA and showing different stiffnesses, obtained by changing their thickness; H9c2 cells (an embryonic myocardium rat cell line) were then cultured onto these substrates. The adhesion properties of H9c2 on these nanofilms were valued by estimating adhesion area and elongation ratio of cells, highlighting similar results to previously reported ones [97]: cells preferentially adhered on rigid substrates in comparison to soft ones. Pensabene et al. [100] used single similar nanosheets to sustain human mesenchymal stem cell adhesion, proliferation, and differentiation, thus highlighting the possibility of using nanosheets as smart carriers of therapeutic cells. Finally, another example in which nanosheets were used as cell culture systems was reported by Ricotti et al. [101]. Here, the development of free-standing single layer PLA nanofilms as cellular scaffolds was described. After their characterization in terms of thickness and roughness, viability, adhesion, and proliferation properties of C2C12 mouse myoblasts cultured onto these substrates were assessed. Early differentiation was also verified.

In conclusion, cell adhesive properties can be tuned by changing both the structural components and the mechanical properties of polymeric nanosheets. Furthermore, the use of suitable biocompatible polymers as well as the choice of the proper cell line should allow the development of novel ultrathin films acting like cell-based matrices to be transplanted in human bodies for the treatment of several heart diseases (such as MI). Such results also suggested the possibility of using polymeric ultra-thin films as structural components for the development of bio-hybrid actuators, opening different and parallel applicative routes with respect to those related to pure regenerative medicine [102].

7. Conclusions

At present, researchers are looking for new alternative strategies to repair the wounded heart and restore its function. Among the several approaches pursued, cell-based treatment is a promising option for cardiac pathologies. In this review, we reported the most recent achievements in the field of cell therapy for MI treatment and heart regeneration. The most common cell sources used in the last decades for cardiac repair and the traditional approaches employed to delivery cells at the damaged site have been described. Then, a series of novel technologies based on recent advancements of bioengineering and tissue engineering have been illustrated, including patches, fragments, and biomaterials. Finally, an original strategy for cardiac repair based on magnetic nanosheets has been discussed, highlighting the tremendous potential and promises that nanoscaffolds have within the therapeutic challenge related to heart regeneration.

On the basis of the findings reported in this last section, the magnetic nanofilm-based approach looks really promising. This therapeutic philosophy implies that the injection of cell-seeded nanosheets inside the body and its guidance up to the damaged heart site can deeply improve MI therapeutic outcomes. Furthermore, the possibility of loading the nanofilm with specific growth factors or drugs makes this new bioengineering approach even more appealing and promising.

Conflict of Interests

Letizia Ventrelli attests to the fact that all authors listed do not have any financial relation with the commercial identity mentioned in this paper, and there is no conflict of interests.

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