Journal of Controlled Release 365 (2024) 617–639

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review article

Nanomaterials-combined methacrylated gelatin hydrogels (GelMA) for cardiac tissue constructs

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ARTICLE INFO

Keywords:
GelMA hydrogel
Nanomaterials
Carbon nanotubes
Cardiac tissue repair
3D bioprinting

ABSTRACT

Among non-communicable diseases, cardiovascular diseases are the most prevalent, accounting for approximately 17 million deaths per year. Despite conventional treatment, cardiac tissue engineering emerges as a potential alternative for the advancement and treatment of these patients, using biomaterials to replace or repair cardiac tissues. Among these materials, gelatin in its methacrylated form (GelMA) is a biodegradable and biocompatible polymer with adjustable biophysical properties. Furthermore, gelatin has the ability to replace and perform collagen-like functions for cell development *in vitro*. The interest in using GelMA hydrogels combined with nanomaterials is increasingly growing to promote the responsiveness to external stimuli and improve certain properties of these hydrogels by exploring the incorporation of nanomaterials into these hydrogels to serve as electrical signaling conductive elements. This review highlights the applications of electrically conductive nanomaterials associated with GelMA hydrogels for the development of structures for cardiac tissue engineering, by focusing on studies that report the combination of GelMA with nanomaterials, such as gold and carbon derivatives (carbon nanotubes and graphene), in addition to the possibility of applying these materials in 3D tissue engineering, developing new possibilities for cardiac studies.

Abbreviation: 3D, Three-dimensional; ACE, Angiotensin-converting enzyme; AMI, Acute myocardial infarction; AuNPs, Gold nanoparticles; AuNWs, Gold nanowires; CAD, Coronary artery disease; cECM, Cardiac extracellular matrix; CJS, Centrifugal jet spinning; CMs, Cardiomyocyte; CNT, Carbon nanotube; CTAB, Cetyltrimethylammonium bromide; CVDs, Cardiovascular diseases; Cx-43, Connexin-43 protein; EBs, Embryoid bodies; ECM, Extracellular matrix; EY, Eosin yellowish; FAK, Focal adhesion kinase; fGO, Graphene oxide nanosheets; fGOVEGF, fGO nanosheets and pro-angiogenic vascular endothelial growth factor-165; GAG, Glycosaminoglycan; GelMA, Gelatin methacrylate; G-GNR, GelMA-coated GNRs; GNRs, Gold nanorods; GO, Graphene oxide; hCPCs, Human cardiac progenitor cells; hPSCs, Human pluripotent stem cells; Irgacure 2959, 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone; LAP, Lithium phenyl-2,4,6-trimethylbenzoylphosphinate; MA, Methacrylic Anhydride; MeHA, Methacrylated hyaluronic acid; μCOP, Microscale Continuous Optical Printing; MMP, Matrix metalloproteinases; MWCNTs, Multi-walled carbon nanotubes; NMVCMs, Neonatal mouse cardiomyocytes; NVP, N-vinyl pyrrolidone; NPs, Nanoparticles; NRs, Nanorods; ODEX, Oxidized dextran; PDA-rGO, Graphene Oxide with Polydopamine Reduction; PEGDA, Poly(ethylene glycol) diacrylate; RGD, Arginine-glycine-aspartic; rGO, Reduced graphene oxide; SMCs, Smooth muscle cells; SPR, Surface plasmon resonance; SWCNTs, Single-walled carbon nanotubes; TEOA, Triethanolamine; TGF- β1, Transforming growth factor-β1; VA-086, azobis[2-methyl- N- (2 -hydroxyethyl)propionamide; VEGF, Pro-angiogenic vascular endothelial growth factor-165; VICs, Valvular interstitial cells.

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https://doi.org/10.1016/j.jconrel.2023.11.056
Received 3 August 2023; Received in revised form 21 November 2023; Accepted 28 November 2023
Available online 7 December 2023

1. Introduction

Cardiovascular diseases (CVDs) are one of the leading causes of morbidity and mortality worldwide. These diseases affect the heart and blood vessels, with the most common cause being atherosclerosis, which leads to lesions and blockages in the coronary arteries. Among these diseases is acute myocardial infarction, commonly known as a heart attack, which occurs due to the obstruction of blood flow to the heart [1]. According to the World Health Organization (WHO), CVDs are the primary cause of death globally, and a portion of these deaths is attributed to heart attacks. Several risk factors contribute to CVD, including high blood pressure, smoking, diabetes mellitus, and lipid abnormalities [2,3].

These CVDs frequently result in pathological changes that affect the structure and function of the heart, such as in the cases of acute myocardial infarction and heart failure. This is primarily attributed to the lack of natural endogenous cardiac stem cells in adults, which prevents local regeneration and leads to prolonged and irreversible damage. Moreover, the existing therapeutic approaches focus on alleviating symptoms rather than repairing cardiac tissue, often requiring transplants that carry a significant risk of rejection [4].

Despite conventional treatment with medicines, tissue engineering emerges as a potential alternative for advancing and treating these patients. Cardiac tissue engineering aims to restore, the contraction, and pumping function of the heart by utilizing biomaterials implanted at the site of injury [5]. Thus, in recent years, the current goal of medicine is to use biomaterials to replace or repair tissues, mainly cardiac, through endogenous regeneration. Thus, these biomaterials must have the characteristics of biocompatibility, with good mechanical and biodegradable properties [6].

Hydrogels have been increasingly used as cell support, due to their similarity with the characteristics of the extracellular matrix (ECM), support for cell growth, and tissue formation [7]. ECM is produced with synthetic or natural materials and it has been used to stimulate cell growth while maintaining physical properties, such as mechanical resistance, degradation, swelling, and strength [8]. Furthermore, biopolymer hydrogels have received attention due to their versatility, biocompatibility, and *in vivo* degradation capacity [9]. Thus, several biopolymers such as gelatin, fibroin, chitosan, collagen, and fibrin have been growing, as they can support cell adhesion [10]. Among these materials, gelatin in its methacrylate (GelMA) form stands out. Gelatin is a biodegradable and biocompatible polymer with tunable biophysical properties and, when applied to gelatin hydrogels, these properties can be viable for various cell types. In addition, gelatin has the ability to replace and perform collagen-like functions for cell development *in vitro* [11].

Another important consideration is that hydrogels used in cardiac tissue engineering must possess electrical conductivity to enable the transmission of electrical signals between cells and promote the adhesion and contraction of cardiomyocytes. Consequently, the medical field has been exploring the incorporation of nanomaterials into these hydrogels to serve as conductive elements for electrical signaling [12].

Nanotechnology enables the utilization of a diverse array of materials in the biomedical field, with many exhibiting adjustable physicochemical properties such as optics, electronics, and mechanical. Due to its remarkable versatility, these materials find extensive application in bioengineering. Nanomaterials play a crucial role in enhancing the performance of biosynthetic tissues by evaluating their molecular structure and interactions with cellular receptors, thereby facilitating significant advancements in the field [13]. Several studies have already demonstrated the utilization of metallic nanomaterials in regenerative medicine and cardiac tissue engineering [14–16]. Metallic nanoparticles, such as silver (Ag), gold (Au), and copper (Cu), are well-known for their various biomedical and pharmaceutical applications [17].

Use of GelMA hydrogels combined with nanomaterials is growing to enhance responsiveness to external stimuli and improve certain properties of these hydrogels. They are employed in various biological applications, such as cell culture, complex tissue engineering platforms, and delivery vehicles for drugs, genes, or growth factors [18]. Evaluating their application in cardiac muscles, these nanomaterials are necessary to achieve electrical conductivity compatible with that of healthy myocardium, aiming to restore cardiac functions [19].

Thus, this study aims to revise the current state of art regarding the utilization of GelMA hydrogels, with nanocomposites, to promote electroconduction and aid in the recovery of cardiac tissues. This study highlights the uses of GelMA hydrogels with nanomaterials of diverse characteristics, focusing on their potential in the cardiovascular field and prospects for 3D bio-printing. The primary objective is to critically analyze nanomaterials and GelMA hydrogels as versatile substrates for cardiovascular treatment, exploring current challenges and future directions in regenerative action. While GelMA finds various applications, this article specifically investigates the application of GelMA hydrogels and GelMA-based cells associated with nanomaterials that facilitate contraction and electrical conduction, highlighting their current trends in cardiac tissue regeneration.

To understand the relevance of this research topid, a search in the scientific literature was performed. The search was fine-tuned to papers published in English, covering the timeframe between 2010–2023. Fig. 1 shows the bibliometric map of scientific articles indexed on Pubmed Medline data (https://www.ncbi.nlm.nih.gov/pubmed/). The number of papers published about GelMA in the cardiac tissue engineering area has maintained in recent years.

The analysis of bibliometric data of this study was conducted using the VOSviewer software version 1.6.16 [20], using the Pubmed database. The selected keywords for data extraction from titles and abstracts were “GelMA” and “cardiac tissue” and “nanomaterial”. Fig. 1 displays the resulting bibliometric map generated by this analysis. This map emphasizes the significance of cardiac tissue engineering and hydrogels as central concepts within the established relationships.

VOSviewer categorized the map elements into a total of 7 clusters, with the largest cluster, represented in red, comprising 16 items, among them “electric conductivity”, “electric stimulation” and “conductive hydrogels” highlighting the relevance of electrical conduction in studies including the use of GelMA in cardiac tissue engineering. The second largest cluster, depicted in green, contains terms such as “biocompatible materials” and “hydrogel” pointing out the different possibilities of biomaterials that can be associated with tissue engineering. It is also worth mentioning another group of concepts related to the new possibilities of using nanomaterials in cardiac tissue engineering, consisting of terms such as “nanogels”, “nanocomposites” and “nanomaterial”.

2. Epidemiology of cardiovascular diseases and current treatments

The United Nations aims, through the Sustainable Development Goals focused on health and well-being, to reduce by one-third the mortality from non-communicable diseases through prevention and treatment by 2030 [21]. Among these diseases, cardiovascular diseases are the most prevalent, accounting for approximately 17 million deaths annually, with the highest rates found in low- and middle-income countries [22,23].

CVDs are a group of disorders affecting the heart and blood vessels. They encompass coronary heart disease, cerebrovascular diseases, rheumatic heart disease, deep vein thrombosis, pulmonary embolism, and peripheral arterial diseases. These diseases are non-communicable, widespread, and the leading cause of death worldwide [24].

Several risk factors have been established for CVDs, including: (i) hypertension, (ii) obesity, (iii) smoking, (iv) diabetes mellitus, (v) dyslipidemia, (vi) sedentary lifestyle, (vii) alcohol consumption, and (viii) poor diet. These are known as modifiable risk factors (Fig. 2) [25]. Additionally, there are also risks related to sex, race, genetic predisposition to coronary diseases (family history), and age, which are non-

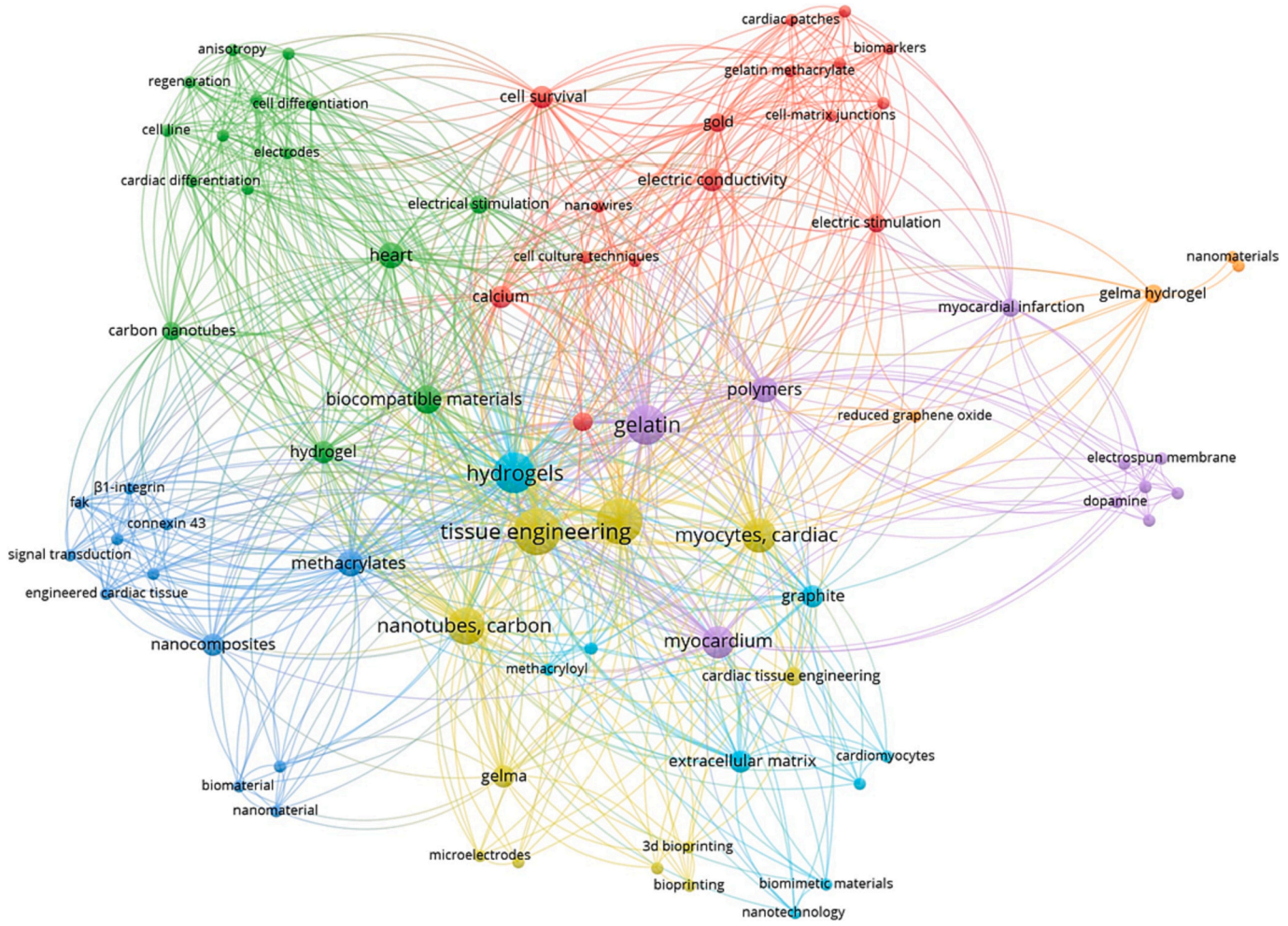

Fig. 1. Bibliometric map using PubMed data published between 2010 and 2023, searching for the keywords "GelMA" and "cardiac tissue" and "nanomaterial", created with VOSviewer software version 1.6.16 [20].

modifiable risk factors and are inherently linked to the individual [26,27].

Cardiovascular diseases (CVDs) not only affect the quality of life of patients but also generate high costs for health systems. It is estimated that the economic cost of CVDs is directly associated with hospitalizations and complex surgical procedures, with the cost of hospitalization accounting for 70% of the total cost of treating patients with CVDs. This represents a significant challenge for health systems around the world [28].

Among these CVDs, cerebrovascular accident (CVA) occurs due to impaired blood circulation in the brain as a result of ischemia in an area of the brain. It is responsible for causing disability and early treatment can reduce the associated morbidity and mortality [29,30]. Cerebrovascular diseases in 2020 were responsible for more than 7 million deaths [31].

Rheumatic heart disease, on the other hand, is an aggravation of rheumatic fever, which is responsible for generating valvular damage caused by an abnormal immune response to infection by group A streptococci. This damage can be irreversible in the valves and lead to heart failure. In addition, it is responsible for cardiovascular death in children and young adults in countries where medical care is inadequate [32–34]. Given this, around 2% of deaths from cardiovascular diseases worldwide are related to rheumatic heart disease [35].

In addition, cogenetic heart disease (CHD) is caused by a congenital defect that leads to an abnormal condition in the structure or function of the heart and affects around 1% of newborns [34,36]. CHD develops after birth and its first symptoms are seen until the first years of life, in addition many of these heart defects are part of genetic and chromosomal syndromes [37].

Coronary artery disease (CAD) is a cardiovascular disease characterized by atherosclerotic occlusion of the coronary arteries. This condition results in the narrowing or blockage of the coronary arteries, which can lead to the sudden and complete interruption of blood flow, with Acute Myocardial Infarction (AMI) being a severe manifestation of this condition. CAD is associated with approximately 17.8 million deaths annually, representing one of the leading causes of death related to cardiovascular diseases [38].

AMI, also known as a heart attack, is responsible for a large number of deaths worldwide, with a prevalence of around three million people affected. Another important factor is the high out-of-hospital mortality rate: a third of patients die before reaching the hospital and around 50% die on admission [39,40].

The severity of the infarction is directly linked to the formation of scars at the infarct site, resulting in altered functional behavior of the heart due to the production of scar tissue, altering cardiac function. Cardiac damage in adults, often caused by AMI, leads to heart failure, as the lesion causes irreversible loss of cardiomyocytes, along with the absence of cardiac stem cells to aid tissue recovery [41]. As the lesion progresses, the site undergoes remodeling associated with fibrosis, leading to ventricular dysfunction [42].

Thus, tissue healing after AMI results in altered excitation-contraction coupling, affecting the conduction of electrical signals and

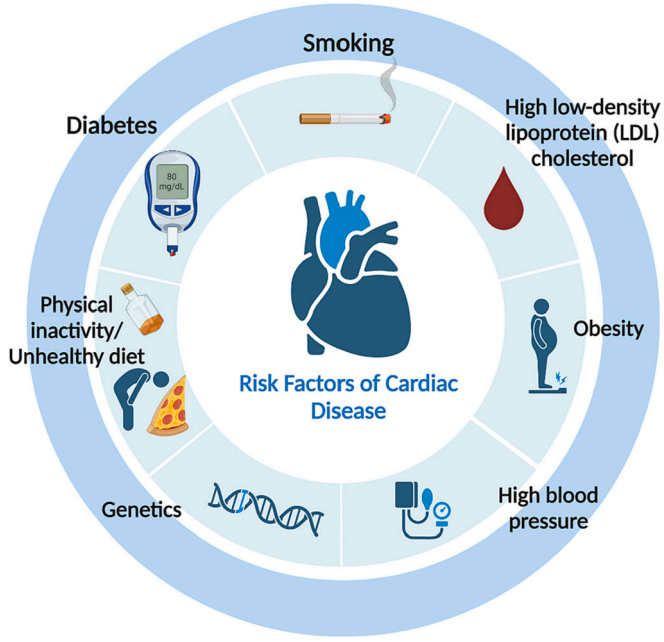

Fig. 2. Main cardiovascular disease risk factors: behavioral risk factors are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol and underlying determinants of CVDs as age, poverty, stress and hereditary factors.

the contraction of the cardiac muscle [43,44]. As a result, the formation of scar tissue leads individuals to experience arrhythmias or permanent changes in cardiac ejection [45]. Patients who survive an AMI can develop heart failure as a late complication, as the loss of functional cardiomyocytes compromises the heart's ability to pump blood [46].

Heart failure affects approximately 26 million people worldwide and can be considered a global pandemic [47,48]. Patients with complications such as dysrhythmia and heart failure following an acute myocardial infarction constitute a subgroup of individuals at high risk of morbidity and mortality [49].

Heart rhythm disorders (arrhythmias) frequently occur during and shortly after myocardial infarctions, and these arrhythmias often lead to early death [50]. Ventricular arrhythmias associated with myocardial infarction are one of the main causes of cardiovascular mortality and require better prevention and treatment [51].

Ventricular arrhythmias (VA) can manifest from asymptomatic to cardiac arrest and sudden cardiac death (SCD), thus standing out as a common cause of SCD in relation to myocardial infarction (MI) and heart failure [52]. Heart failure often results in cardiac arrhythmias, which can lead to cardiomyopathy such as left ventricular systolic dysfunction (LVSD), in which rapid and/or irregular ventricular rate occurs [53].

Current therapeutic approaches for treating AMI aim to restore blood supply to cardiomyocytes through medication, percutaneous coronary intervention, and coronary artery bypass surgery [54]. Despite their effectiveness, these techniques are limited by the potential for severe complications for the patient [55]. Despite the advantages of AMI treatment, there is no treatment capable of preventing the loss of cardiomyocytes or post-AMI tissue remodeling [56].

In addition to the loss of functional cardiomyocytes, increased workload on the heart can lead to progressive cardiac dysfunction and heart failure [57]. In order to prevent ventricular failure, drug therapies also include the use of angiotensin-converting enzyme (ACE) inhibitors and beta-blockers [44,54]. However, since the focus of these treatments is mainly on symptoms without cardiac repair, they are unable to restore the cardiomyocytes lost due to fibrosis [58].

Thus, the loss of functional tissue reduces the pumping capacity of the heart due to post-AMI cardiac remodeling, leading to heart failure [46]. In this context, heart transplantation is considered a last resort in severe cases [59]. However, the number of donors is extremely limited, in addition to the high cost and risk of rejection [28,60].

With the need related to CVDs, current medicine seeks alternative therapies to assist in the recovery of cardiac tissue. Injectable hydrogels, for example, are prominent in cardiac tissue engineering as they allow minimally invasive delivery, reducing the risk of complications associated with traditional surgical approaches [4,5].

Studies have increasingly investigated the use of cardiac stem cells to stimulate endogenous regeneration [61,62]. Additionally, the application of genes through nanotechnology [63], the use of scaffolds and hydrogels in bioengineering [64,65], and the utilization of 3D bioprinted structures [66–68] have been studied as promising approaches to assist in the regeneration and restoration of cardiac function in cases of myocardial injury. These approaches aim to repair damaged tissue and have the potential to represent innovative alternatives for the treatment of acute myocardial infarction.

3. Regenerative medicine and cardiac tissue engineering

Tissue engineering and regenerative medicine aim to construct scaffolds that act as a matrix, aiding in cellular organization, viability, and tissue formation [69,70]. With the increasing number of people affected by cardiovascular diseases worldwide, there is a need to develop more effective and specific treatments to alleviate this situation. Cardiac tissue engineering, therefore, emerges as an alternative to invasive treatments or heart transplantation [71].

Regenerative medicine aims to repair, replace, or regenerate damaged tissues and organs, providing an alternative approach to the advancement and treatment of patients with heart injuries. The materials used in regenerative medicine are designed to support cell distribution and growth, both *in vitro* and/or *in vivo*. These materials should eventually degrade and be non-toxic, eliminating the need for further removal [72]. By utilizing biomaterials, regenerative medicine helps restore heart contractions and pumping, ultimately improving the quality of life for affected individuals [73].

Some studies apply cardiac cells in traditional two-dimensional (2D) cell culture models; however, these cells lose intrinsic common characteristics. Thus, they influence the ability to respond to physiological stimuli, presenting a regression of functional and structural characteristics, tending to have a less mature phenotype, so that, consequently, current 2D systems often do not reproduce reliable results to the *in vivo* response [74].

Cell therapy, based on stem cells, is emerging as a promising prospect in regenerative medicine. However, its application encounters significant challenges, such as low cell retention and survival, especially after *in vivo* administration. That makes cell therapy difficult, especially if applied in the acute phase of AMI, which can help the reduction of the death of SMCs and aid repair. However, most cells die within the first 24 h, often due to immune reaction, poor oxygenation, free radicals, and inflammatory cytokines [75].

In the field of cardiac cell studies, human pluripotent stem cells (hPSCs) stand out due to their exceptional ability to differentiate into various cell types [76]. Effective differentiation protocols allow these cells to transform into cardiac cells (CMs), along with vascular endothelial cells (ECs) and vascular smooth muscle cells (SMCs). These specialized cells become the focus of application for the production of attractive *in vitro* cardiac tissues for the study of tissue engineering and regenerative medicine [77].

Despite this possible application of these cells, these *in vitro*-generated SMCs often show electrical immaturity due to the proliferation of fetal SMCs. This occurs because the cells are small in size, and the sarcomere structures are disorganized with poor excitability and contractility [78]. However, science is progressing to overcome these immaturity problems and promote the maturation of SMCs, using techniques, such as electrical stimulation, three-dimensional (3D)

cultures, and the development of artificial cardiac tissues, such as hydrogels that simulate the extracellular matrix (ECM), with the greatest emphasis on cardiac tissue engineering to provide a means of maturation, contractile function, and electrical stimulation [79].

Studies, such as that from Kerscher et al. (2016) [80], show that polymer hydrogels (e.g., GelMA) can be used to encapsulate hiPSCs and support 3D differentiation to generate uniformly contracting cardiac tissues. In this study, the encapsulated cells were evenly distributed and maintained a high viability rate of over 75% in the hydrogels. In addition, it efficiently facilitated cardiac growth and differentiation, resulting in a significant increase in the frequency and speed of spontaneous contractions, demonstrating the importance of structures for cell support.

Thus, more and more 3D structures are being formed in an organized and functional way, given the need to resemble the complexity of living tissues. This method enables the incorporation of different cell types, ECM-like materials and biochemical signals, such as growth and cell remodeling factors [81]. Thus, several 3D structures for cell culture such as scaffold-based (hydrogels, bioprinting), scaffold-free (organoids and spheroids) and organ-in-a-chip have been used in tissue engineering [82].

In cell-based therapy, cardiomyocytes are the main cells studied for this application; however, they have a low integration with the tissue *in vivo* with low cell retention. In addition, there is a lack of precursor cell sources, so that cell therapy and tissue engineering seek an efficient regeneration process, as it is possible to have an appropriate structure with good chemical and mechanical performance resulting in cell interaction-material, showing promise in *in vitro* cultivation before direct delivery of cells *in vivo* [83].

Thus, these biomaterials allow adequate morphological adaptation, associating stem and/or progenitor cells in these materials, obtaining a synergistic action, bringing a capacity for tissue regeneration and reconstruction with the biomaterial acting as a support. In addition, it can be enhanced with the addition of growth factors and other molecules intensifying tissue interaction [84].

It is therefore required the use of structures and materials that support reconstruction and regeneration, thus contributing to cell support, promoting cell adhesion, proliferation and differentiation. It also assists in gaseous and nutrient exchanges, in addition to assisting in the revascularization process [85]. Still, one of the objectives of cardiac tissue engineering is the electrical coupling between cardiomyocytes, which can be improved with the addition of nanomaterials in these produced matrices, which provides electrical conduction capacity and mechanical resistance for cell adhesion and communication [86,87].

4. Hydrogels for cardiac tissue construct

Hydrogels are three-dimensional hydrophilic polymeric network structures capable of absorbing and retaining large amounts of water. With their high water retention capacity, these materials exhibit diverse potential for biological applications [88,89]. Hydrogels have been applied in several areas, as biosensors [90], drug delivery [91], tissue and spinal cord regeneration [92], and as matrices for cells in tissue engineering [93,94].

Hydrogels stand out due to characteristics such as biocompatibility, adjustable mechanical properties and the ability to encapsulate cells and growth factors [95]. Furthermore, they can provide a microenvironment that mimics the extracellular matrix of the heart, allowing the adhesion and migration of cardiac cells, such as fibroblasts and endothelial cells [67]. Additionally, the ability of the hydrogel to adjust to the exact shape of the trauma is a feature that cannot be achieved using conventional scaffolds, opening doors for conducting studies on the use of hydrogels for cardiac tissue engineering [96].

The mechanical stability of the hydrogel is crucial in cell-based applications, as it provides essential support for cells. This material must create an environment that promotes cell adhesion, proliferation, and differentiation, assisting cells in generating their own extracellular matrix (ECM). Consequently, the stiffness of the hydrogel matrix can influence critical properties, such as cell morphology, metabolism, and function within the matrix [97,98].

Furthermore, the water-rich nature of hydrogels plays a crucial role in facilitating electrical signaling within cells, leading to increased excitability, as observed in cardiomyocytes and neurons [56,99]. The hydrogel's ability to swell not only enhances the diffusion of molecules, including nutrients, but also supports cell differentiation, survival, and serves as a facilitator for drug delivery (Fig. 3) [100].

The formation of hydrogels requires the cross-linking of their polymer chains in an aqueous solution. Thus, physical cross-linking (physical gelation) or chemical cross-linking can be used [101]. The physical process is based on the intrinsic properties of the polymer and is easy to obtain and reverse, because it does not modify the polymer chains. In chemical crosslinking, it is necessary to modify the chains of the material through chemical changes, thus allowing the development of a more complex and customized microenvironment, being also a more complex process [102].

Furthermore, hydrogels can be fabricated using a wide range of polymers, which directly influence their porosity and stiffness, thereby enabling customization to fulfill specific application requirements [87]. The primary goal of hydrogels is to offer mechanical support for cell growth and differentiation within a three-dimensional environment, closely mimicking the *in vivo* conditions [103]. There are several types of polymers that can be used in the formation of hydrogels, including natural polymers such as collagen [104], sodium alginate [100], chitosan [105], gelatin [106], hyaluronic acid [107], solubilized decellularized porcine cardiac extracellular matrix (pcECM) [108], fibrin [98], and fibroin silk [109]; and synthetic polymers such as polyacrylic acid (APA) [110], polyethylene glycol [111], poly(N-isopropylacrylamide) [112] and polyvinyl alcohol [113].

Each type of polymer has properties that affect the behavior of the hydrogel, such as its mechanical strength and biodegradability, so the choice of polymer to be used in hydrogel formation depends on the requirements of the intended application [114]. Natural polymers tend to have higher biocompatibility and biodegradability, but may have lower mechanical strength compared to synthetic polymers [115]. Synthetic polymers offer more control over the mechanical properties, reproducibility and modification of the hydrogel, but may have lower biocompatibility. Additionally, combinations of natural and synthetic polymers are also used through hybrid hydrogels to harness the advantages of both types of polymers, resulting in improved mechanical properties, increased biocompatibility, and adjustable degradation rates [116]. Furthermore, chemical modifications of natural polymers can be performed to enhance their application, as is the case with gelatin modified into methacrylated gelatin (GelMA) [87,117].

In this way, photocrosslinkable hydrogels obtained through chemically modified materials have emerged with the aim of enhancing the adhesion of the material to tissues. These hydrogels improve upon the biocompatibility and mechanical properties of natural polymers while allowing for the delivery of the material in liquid form, solidifying only at the site of application [118]. Among these photocrosslinkable hydrogels, some notable ones are based on poly(ethylene glycol) diacrylate (PEGDA), gelatin methacryloyl (GelMA), and methacrylated hyaluronic acid (MeHA), which are widely used in tissue engineering [119].

In order to produce cardiac tissues, it is necessary to carefully evaluate several aspects, such as the arrangement and alignment of cardiomyocytes. The production of a dense tissue and the presence of a vascular network are required to facilitate gas exchange, to provide nutrients and promote the natural integration of blood vessels, thus forming a new pathway for blood circulation [13,120].

Therefore, to successfully create a cardiac tissue capable of performing its cardiac functions, it is essential to utilize biomaterials with specific characteristics suitable for biological application [84].

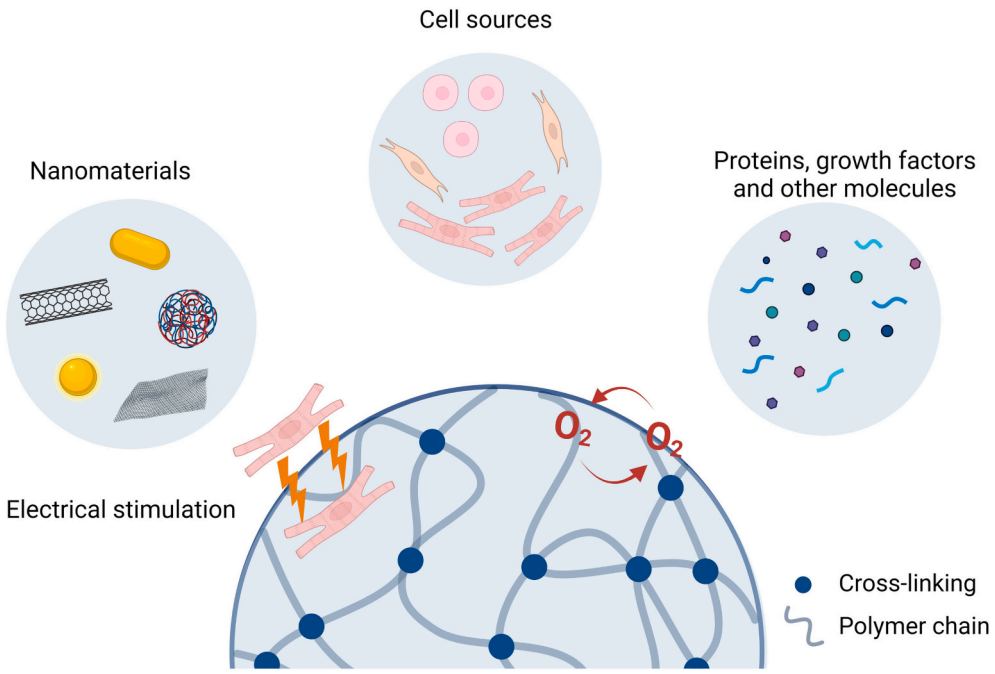

Fig. 3. Illustrative scheme identifying potential applications of hydrogels in tissue engineering. It is also possible to add growth factors and other molecules that will help the material's communication and cell adhesion. Nanomaterials may also stimulate the electrical communication between cells, which is fundamental for tissues such as neural and cardiac.

Furthermore, it is important to avoid potential arrhythmias when introducing the biomaterial. To address this concern, electrically conductive materials can be utilized, which possess properties that meet the requirements of cardiac tissue [121].

Hydrogels incorporated with inorganic nanoparticles have been used to modify the properties of scaffolds for tissue engineering applications. Conductive materials, such as gold nanoparticles (AuNPs) [122], carbon nanotubes (CNTs) [1,123–125], graphene [56], and conductive polymers, are added to polymers to create a biocompatible and conductive material for biomedical applications [126,127].

For the application of these hydrogels in cardiac treatment, it is necessary to have properties similar to the native myocardium, such as good elasticity and appropriate mechanical properties to support heart contraction. Furthermore, for the treatment and recovery of damaged tissues, such as in the case of myocardial infarction (MI), the electrical conductivity of these hydrogels is crucial [56]. Additionally, the incorporation of nanomaterials helps to enhance cellular sensitivity and contributes to ventricular synchrony, preserving cardiac contraction function and promoting angiogenesis [128].

5. Gelatin methacrylate (GelMA)

Gelatin methacrylate (GelMA) is known to be a photocrosslinkable biomaterial widely used in tissue engineering because it has appropriate strength, which maintains functional bioactivity. The use of gelatin in this field has aroused interest in research due to the characteristics that this material presents, such as biocompatibility, biodegradability, viscosity, flexibility, in addition to easy handling and low cost [73].

The first reports on methacrylated gelatin were made through studies by van den Bulcke et al. (2000) [129]. They described the production of a hydrogel based on the modification of gelatin with methacrylic anhydride forming a chemically stable material that is able to be crosslinked with UV irradiation after the addition of a photo-initiator. Thus, a modified gelatin chemical network is obtained. In this study, the effect of the degree of substitution of the methacrylic groups, the concentration of polymer, the concentration of photo-initiator, and the UV irradiation time, as well as the storage conditions were evaluated. With this study, it was possible to highlight GelMA as an attractive material for biomedical applications, due to its chemical cross-links being well controllable, its structural capacity is adequate according to the degree of substitution chosen, expanding the field of study for the application of GelMA in several areas [129].

Gelatin is a natural protein obtained from collagen. It can be obtained through two distinct methods: the alkaline method and the basic pre-treatment method of collagen. These methods aim to weaken the structure of the collagen protein, thereby breaking intermolecular cross-links [18]. Type A gelatin is obtained through acid pre-treatment. In this method, the amide groups present in the gelatin structure, such as asparagine and glutamine, do not undergo hydrolysis, remaining present in the chain. In type B gelatins, alkaline pre-treatment is carried out; in this process, the amide groups undergo hydrolysis forming carboxylic groups (aspartate and glutamate). Another difference between the types of gelatins used is the isoelectric point; type A gelatins have an isoelectric point of 9.4 and type B gelatins of 4.8. This is because the gelatin obtained by the basic method has larger carboxyl groups, decreasing its isoelectric point [130]. Gelatin has chemical groups in its side chains, such as –OH, –COOH, –NH2, –SH. These groups allow the structural modification of gelatin by adding other specific groups in order to improve the deficiencies that gelatin presents [19].

Therefore, methacrylic anhydride (MA), a widely utilized organic compound in polymer synthesis, is employed for the formation of GelMA. It functions by replacing amine groups in gelatin chains with methacryloyl groups, facilitating the modification process [100]. GelMA has the ability to form a hydrogel through photopolymerization due to the formation of methacryloyl groups during its chemical modification with methacrylic anhydride [100]. In the synthesis of GelMA, both type A and type B gelatins can be utilized, ensuring biocompatibility with the methacrylation process [131].

This process is responsible for producing methacrylate gelatin, or photoreactive gelatin methacrylate (GelMA), which can be crosslinked through photopolymerization. This involves the use of photo-initiators and exposure to UV light, which triggers the polymerization reaction and leads to the formation of a hydrogel structure (Fig. 4) [43]. GelMA hydrogels typically contain less than 5% methacrylic anhydride (MA);

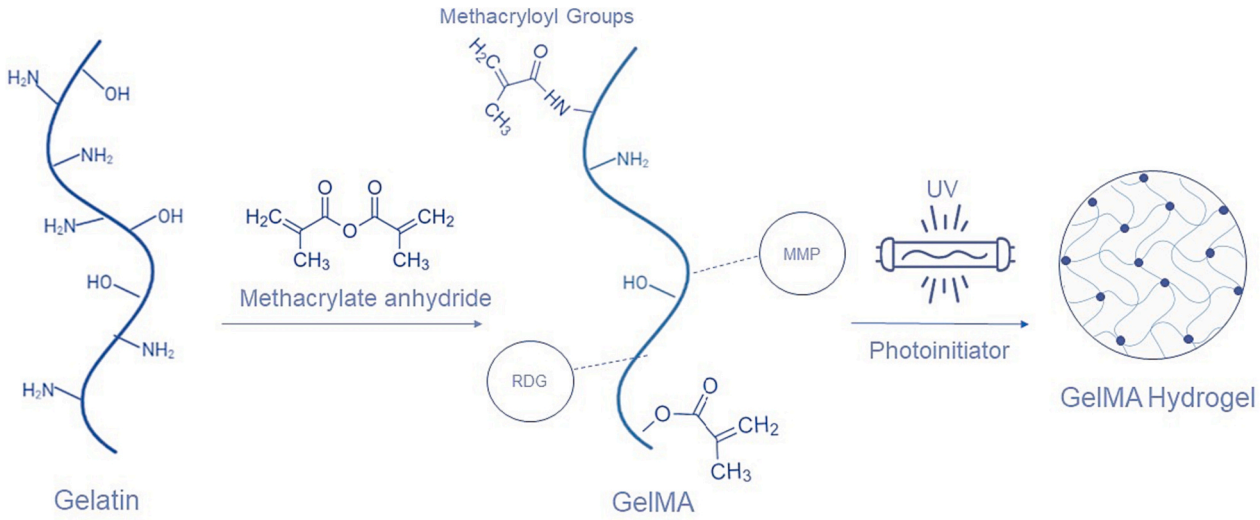

Fig. 4. Synthesis of methacrylated gelatin (GelMA). The primary amine groups of gelatin react with methacrylic anhydride (MA) to add methacryloyl pendant groups. Bioactives from gelatin, such as arginine-glycine-aspartic (RGD) and matrix metalloproteinases (MMP), are still kept in the GelMA chain (first reaction). To obtain the hydrogel, GelMA is crosslinked using UV irradiation in the presence of a photoinitiator (second reaction).

this concentration allows the introduction of methacrylate groups onto the gelatin backbone, enabling the crosslinking process [132–134].

To facilitate the photopolymerization of GelMA and enable gelation and hydrogel formation, specific photoinitiators are employed. The photopolymerization process involves exposing the photoinitiator to specific wavelengths of light, which energize it and result in the generation of free radicals. These free radicals then initiate the formation of a polymeric network. During UV-induced photopolymerization, light triggers the homolysis of chemical bonds within the photo-initiator, creating free radicals that propagate along the polymeric chain. These free radicals subsequently bind to the methacryloyl groups present in GelMA [18]. This modification increases the melting point of gelatin and thus makes it attractive for biological application [135]. However, it is necessary to evaluate the intensity and time of exposure to this light, as these free radicals can also react with cellular components, as happens in the case of hydrogels loaded with cells. The free radicals produced can react with the main components of living cells, such as proteins and nucleic acids, which can affect the condition and viability of cells [136]. Nevertheless, with a reduction in the amount of photoinitiator and lamp intensity, they become compatible [18].

Among the photoinitiators available on the market, there is 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone (Irgacure 2959) and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP). In addition, some studies have used azobis[2-methyl-N-(2-hydroxyethyl)propionamide] (VA-086) for GelMA hydrogels [137,138]. More and more pigments are also being used as photoinitializers, such as eosin yellowish (EY), which has been increasingly studied due to its low toxicity, EY is normally used in a two-component system, with EY as the photosensitizer and triethanolamine as the initiator [139,140]. Among these, Irgacure 2959 is the most widely used in GelMA hydrogel-based studies and photopolymerizes the hydrogel only under UV irradiation at a wavelength in the 360–405 nm range (Table 1).

GelMA hydrogels combine advantages of natural and synthetic hydrogels widely applied in tissue engineering [153]. This is because GelMA has favorable characteristics, such as excellent cell adhesion, biocompatibility and modifiable mechanical properties [154]. An important advantage associated with the application of GelMA hydrogels is the ability to adjust their stiffness according to the application. Thus, the degree of methacrylation can be modified, by changing the structure of the polymer for study purposes, the concentration of the photoinitiator and also the intensity and exposure of the hydrogel to UV light, factors that directly influence the mechanical properties [128,145].

The presence of bioactives, such as arginine-glycine-aspartic (RGD) and matrix metalloproteinases (MMP), makes gelatin an ideal choice for cell development, as these compounds promote cell adhesion and growth, in addition to helping cell remodeling. GelMA hydrogel is highly hydrophilic and favors cell adhesion, with excellent biofunctionality and mechanical tuneability, which makes it a promising material for tissue engineering applications [153].

GelMA possesses binding sites distributed throughout its polymeric chain, which promotes enhanced cell binding. These binding sites include RGD and MMP regions that remain intact even after gelatin methacrylation. However, when encapsulating cells in hydrogels alongside other biomaterials, it becomes necessary to introduce cellular factors, such as peptides or other cellular components, to facilitate cell binding within this environment. This aspect underscores the benefits of utilizing GelMA in conjunction with cells, as it provides a favorable framework for effective cell attachment and interaction [132].

With these positive characteristics for cell growth, studies such as that of Nichol et al. (2010) [132] explored GelMA as a hydrogel for tissue engineering applications. In their study, the cellular behavior of fibroblasts encapsulated within GelMA was evaluated. In their study a cell-loaded microgel was produced, in it the cells readily bound, proliferated, elongated, and migrated when seeded onto GelMA substrates, thus GelMA becomes an attractive material for the creation of cell-loaded microtissues [132].

Thus, with the positive results of GelMA and its association with cells, several studies have been conducted associating this material with cells, such as chondrocytes for cartilage regeneration [155], neural stem cells (NSC) [156] and bone mesenchymal stem cells (BMSCs) for spinal cord regeneration [148], corneal keratocytes [157], among other types of cells.

Several other works have studied GelMA as a scaffold for cardiac tissue models. A 3D cardiac microtissue was developed with co-culture of cardiomyocytes and cardiac fibroblasts using GelMA hydrogel [141]. Besides its application, GelMA has been used for cardiac models as a bioink for 3D bioprinting in several studies [66,67,98,147]. Koti et al. (2019) [67] used a bioink associated with cardiomyocytes, in which cardiomyocytes and fibroblasts were added and cell survival was evaluated after the bioprinting process [67].

Despite the excellent biological properties of GelMA, research has stood out in the combined use of GelMA with bioactive and functional

Table 1
Examples of photoinitiators available on the market for the polymerization of GelMA hydrogels and their cellular impact.

Photoinitiator	Concentration	Wavelength	Exposure Time	Intensity	Light Type	GelMA concentration	Cellular Impact	References
Irgacure 2959	0.5% (w/v)	360–480 nm	50s	800 mW	UV light	5% (w/v)	Good cellular compatibility, specific expression of cardiac proteins and synchronous contraction were present in the study. Despite this, the impact of exposure time, polymer concentration or photoinitiator was not evaluated.	[141]
	0.5% (*w/v*)	360–480 nm	60s	6.9 mW/ cm^2	UV light	5%, 10%, or 15% (w/v) GelMA of low, medium, or high degree of methacrylation	The 5% (*w/v*) hydrogels demonstrated a remarkable process of stretching, migration and formation of interconnected networks with neighboring cells. This behavior was observed at a slightly slower rate in the 10% and 15% hydrogels, indicating that the increase in hydrogel concentration slowed the process. It is important to note that the hydrogels were produced with high precision in their pattern and maintained high cell viability during the experiment.	[132]
	0.5% (*w/v*)	360–480 nm	10, 15, 30 and 60 s	6.9 mW cm^2	UV light	5, 7, 10 and 15% (*w/v*)	The hydrogels bioprinted on day 1 loaded with cells photopolymerized for 60 s were associated with lower viability than the gels photopolymerized for 15 s, the printing process did not affect the health of the encapsulated cells. The bioprinted material obtained higher proliferation rates than the unprinted ones. The hydrogels showed excellent elasticity, which led to effective bioprinting, and the bioprinted constructs showed high cell viability. Despite this, the impact of exposure time, polymer concentration or photoinitiator was not evaluated.	[142]
	0.5% (*w/v*)	360–480 nm	15 s, 25 s and 30s	7.2 mW/ cm^2	UV light	5%	As the concentration of the photoinitiator increased, there was a decrease in cell viability. However, cell viability and cell spreading did not vary significantly between the UV exposure times of 30 s, 1 min and 2 min after 24 h of cultivation. After 48 h of cultivation, there was a reduction in cell viability in relation to the UV exposure time.	[143]
	0.05% (*w/v*)	365 nm UV light	10 min	8 mW/ cm2	UV light	10%	Aortic valvular interstitial cells (VICs) were successfully encapsulated. They retained their natural morphology and demonstrated differentiation in response	[144]

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Table 1 (*continued*)

Photoinitiator	Concentration	Wavelength	Exposure Time	Intensity	Light Type	GelMA concentration	Cellular Impact	References
							to transforming growth factor-β1 (TGF-β1). The polymerization process and the GelMA hydrogel platform exhibited high cytocompatibility, ensuring a consistent percentage of live cells viability.	
	0.25% (*w/v*)	360–480 nm	20s	800 mW	UV light	5%, 7%, and 10% (m/v)	GelMA hydrogel characteristics affect cell spreading but not viability, a higher cellular spreading is expected due to a lower methacryloyl modification degree. Across all experimental conditions, the percentage of live cells was higher than 84%.	[145]
	0.5% (w/v)	365 nm	45 s	2.6 mW/ cm^2	UV light	5% GelMA +2.4 mg/mL of glycosaminoglycan	The fibers produced performed better in the presence of a hydrogel component because it improved the overall viability of the cells, so greater cell viability was observed in the hydrogel samples compared to the pure fiber-only conditions. Despite this, the impact of exposure time, polymer concentration or photoinitiator was not evaluated.	[146]
	0.2%	365 nm	45 s	11 mW/ cm^2	UV light	2% hyaluronic acid glycidyl methacrylate, 2% poly (ethylene glycol), 5%, 10% and 15% GelMA	Promising results on printed material, with long survival times in culture. Cardiomyocyte viability was evaluated on day 3, GelMA with 5% and 7.5% had the highest percentage of viability. GelMA with 5% had greater compatibility. In addition, encapsulated in 5% GelMA also showed positive staining for α-actinin, both for the plate and for parallel lines, throughout the printed scaffold.	[147]
LAP	0.1%	405 nm	20 s	30 mW/ cm^2	UV light	GelMA at 50 mg/mL with different ODEX concentrations of 0, 50 and 70 mg/mL.	Hydrogel with good cytocompatibility and cellular delivery capacity. Despite this, the impact of exposure time, polymer concentration or photoinitiator was not evaluated.	[148]
	0.4%	405 nm	120 s.	10 W	LED	Hydrogel: MeTro (0, 7.5 and 15%) and GelMA (0, 7.5 and 15%). Bioink with: 7.5% MeTro/ 7.5% GelMA, 20% gelatin, 10% GelMA and 23% gelatin	Good cell viability after bioprinting, endothelial barrier function and spontaneous beating of cardiac muscle cells. The printing process supported cellular growth and proliferation without affecting the cells' phenotype. Despite this, the impact of exposure time, polymer concentration or photoinitiator was not evaluated.	[15]
	0.5%	365 nm	5 min	Between 2300 μW/	UV light	0.5% Nanogel functionalized with	Not specified	[149]

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Table 1 (*continued*)

Photoinitiator	Concentration	Wavelength	Exposure Time	Intensity	Light Type	GelMA concentration	Cellular Impact	References
				cm^2 and 1100 μW/cm^2		metacriloil /10% GelMA and 10% GelMA as control		
	0.5%	405 nm	120, 300 and 600 s	7 mW/cm^2	UV light	10%; 15%; 20% e 25% (w/v)	Exposure times of 600 s to UV light resulted in cessation of cell proliferation and almost complete cell death, while exposure times of less than 300 s had minimal effect on cell viability and proliferation.	[67]
	0.1 mM EY; 1.5% (m/v) TEOA; 1% (m/v) VC	450–550 nm	180 s	100 mW/cm^2	Blue-green light	10% (*w/v*)	*In situ* photocrosslinking of GelMA hydrogels using visible light demonstrated the safety and feasibility of delivering these hydrogels in the *in vivo* study. Cell viability remained above 90% in the *in vitro* culture. In addition, the physical properties of the hydrogels, such as mechanics, swelling behavior and porosity, can be adjusted by varying the concentrations of the photoinitiators. Increasing the concentrations of VC and TEOA influences the dynamics of the reaction, resulting in a higher cross-linking density, which can increase the rigidity of the hydrogel, reducing the swelling rate and pore size. It was found that increasing the concentrations of the cross-linking reagents had a negative effect on cell viability in three-dimensional cell cultures. Although the increase improved the mechanical properties of the hydrogel, it affected cell survival. The concentration of EY was shown to have a positive impact on Young's compressive modulus and pore size, while it negatively affected the rate of mass expansion and cell viability. On the other hand, increasing the concentration of GelMA contributed to improving the compressive Young's modulus and cell adhesion. In addition, significant cell proliferation and the successful formation of a 3D cellular network were observed on the fifth day. Low-density GelMA hydrogels supported high cell viability after encapsulation, tissue growth, high-efficiency cardiac differentiation, and increased frequency and speed of spontaneous contraction.	[139]
Eosin Y used in combination with triethanolamine (initiator), 1-vinyl-2 pyrrolidinone and N-Vinylcaprolactam (comonomer)	0.005–1 mM EY; 0.05–5% (*w/v*) TEOA; 0.05–5% (w/v) VC	490–510 nm	0,5, 1, 2, 4, 7 e 10 min	20 mW/cm^2	LED	5–20% (*w/v*)		[140]
	0.01 mM EY; 0.1% (w/v) TEOA and 37 nM NVP	Not specified	10–20 min	48.6 mW/cm^2	visible light	10%, 15%, and 20% (*w/v*)		[150]
	10 mM EY; 1.5% (*v*/v) TEOA and 3.96 μl/mL NVP	Not specified	40s	203 mW/cm^2	visible light	15 mg/mL		[80]

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Table 1 (*continued*)

Photoinitiator	Concentration	Wavelength	Exposure Time	Intensity	Light Type	GelMA concentration	Cellular Impact	References
	1.5%	385 nm	5 min	2 mW/cm2	ultraviolet LED	5, 7.5, 10%, with high and low methacrylation	The concentration of gelatin influenced the specific metabolic activity of the cells and their viability, and with GelMA 5% the cells were more viable and metabolically active. The cell behavior and viability results after 7 days showed that greater functionalization of the gelatin resulted in environments more suitable for prolonged use in 3D. The softer GelMA hydrogels were shown to improve cell-cell interactions when cocultured.	[151]
VA-086							With tests prepared using either VA086 (0.75% w/v) or Irgacure (0.05% *w/v*), the viability exceeded 80% on the initial day for all three cell types when subjected to the minimal crosslinking required. In a hydrogel 3D environment, Irgacure yielded superior mechanical advantages with less effects on encapsulated cells when contrasted with VA086. Furthermore, the findings suggested that higher concentrations of photoinitiator radicals in the encapsulation conditions correlated with lower cell viability, despite this, the impact of each exposure time, polymer or photoinitiator concentration on cell viability have not been investigated.	
	0.25–1.0% *w/v*	365 nm	3, 5, and 7 min	2 and 136 mW/cm2	LED array	20% *w/v* (GelMA/PEGDA)		[152]

Captions: EY, Eosin Yellowish; Irgacure 2959, 2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone; LAP, lithium phenyl-2,4,6-trimethylbenzoylphosphinate; LED, light-emitting diode; NVP, 1-vinyl-2 pyrrolidinone; ODEX, oxidized dextran; PEGDA, polyethylene glycol diacrylate; TEOA, triethanolamine; VA-086, azobis[2-methyl- N- (2 -hydroxyethyl)propionamide]; VC, N-vinylcaprolactam.

nanomaterials, with the purpose of enabling physicochemical and biological properties to the hydrogel [93]. This association has benefits, such as viscoelastic characteristics, which allow 3D printing of bioinks, conductivity for cells with the aim of repairing specific tissues, in addition to providing therapeutic capacity in the tissue repair process [18,158]. The development of cell-loaded electroactive hydrogels has gained prominence in tissue engineering research due to their inherent electrical properties that can induce the regulation of cell behavior, in which stimuli can promote the growth and proliferation of electro-responsive cells [159].

Corroborating this, several studies report the application of GelMA associated with metallic nanomaterials; however, most of the studies for cardiac application are still limited to gold, graphene, and carbon nanotube-based nanomaterials (Table 2). In the study by Li et al. (2020) [160], gold nanowires were incorporated into GelMA hydrogels to construct cardiac tissue. In this study, the authors obtained positive results on the viability of the CMs and demonstrated electrical and mechanical properties with beating activity, making it a promising biomaterial. Even as the use of carbon-based materials, like graphene reported in the study by Zhu et al. (2022) [148], conductive hydrogel networks were successfully produced with GelMA and oxidized dextran (ODEX) and rGO (reduced graphene oxide), combining the electrical conductivity of graphene oxide. These materials have been developed and used as biological substitutes, a fact that triggers predictable biological responses [161].

6. Nanomaterials-combined GelMA for cardiac disease

The incorporation of nanocomposites based on inorganic nanoparticles into hydrogels is receiving significant attention as a strategy to engineer inorganic-organic structures with exceptional mechanical and biological properties. This approach is dedicated to maximizing the overall performance of these hybrid materials, ushering in unprecedented possibilities for cutting-edge applications in the realm of regenerative medicine. By pushing the boundaries of performance, it opens up new horizons and opportunities to revolutionize the field, ultimately enhancing our ability to address complex tissue regeneration challenges with remarkable precision and efficacy [175].

Several nanoparticles, associated with cardiac studies, are currently synthesized from metals (e.g., silver [176], gold [107,160], and iron

Table 2
Studies describing the use of GelMA associated with nanomaterials for cardiac studies.

Material	Used nanomaterial	Physical Response	Biological response	References
GelMA Hydrogel	CNTs (multiple walls)	The CNTs improved the mechanical stability of the hydrogel. The CNT-GelMA exhibited much better mechanical integrity, supporting tissue contraction.	After cell seeding, it was possible to visualize homogeneous and interconnected cells covering the entire surface area of CNT-GelMA. It exhibited cellular adhesion, spreading, retention, and viability. Furthermore, no cytotoxic effects of CNT were observed during the 7 days of the evaluation period.	[162]
GelMA Hydrogel	GO nanosheets are functionalized with polyethyleneimine (PEI) complexed with VEGF DNA (pro-angiogenic gene).	The addiction of fGO significantly increased the viscosity of the hydrogels, as well as improved their mechanical strength.	The graphene-containing hydrogel showed a significant increase in gene delivery efficiency compared to hydrogels that did not contain GO (pure GelMA hydrogel and GelMA hydrogel + free DNA). Additionally, it exhibited greater cell proliferation, and in *in vivo* biocompatibility studies, GO and GelMA did not induce any significant toxicity or inflammatory response. In terms of efficacy, a significant reduction in fibrotic area was observed.	[163]
GelMA and PEG hybrid hydrogels	CNTs	The addition of CNTs to GelMA hydrogel resulted in improved mechanical integrity, allowing for easy probing. The wavy structure of CNTs provided a perpendicular direction to the alignment of nanotubes, providing a sufficient electric field to cardiac cells for stimulation at lower thresholds to induce beating.	The CNT-GelMA layer was deposited on top of the gel substrate embedded with CNTs as a cell adhesive layer, as the CNT electrodes on a PEG substrate were unable to support the adhesion of cardiomyocytes.	[164]
GelMA Hydrogels	Gold nanorods (GNRs)	The GelMA-GNR hybrids exhibited enhanced electrical and mechanical material characteristics.	The association with GNR induced high cell retention and improved cytoskeletal organization, leading to the formation of interconnected layers of cardiac tissue. The tissue exhibited organized, compacted, and uniform architecture while maintaining a high level of cell viability and metabolic activity.	[165]
GelMA hydrogel	Reduced graphene oxide (rGO)	The presence of rGO nanoparticles enhanced the mechanical properties of the hydrogels. The conductivity of the hydrogels can be significantly increased by incorporating conductive rGO sheets.	There was increased cell adhesion to the hybrid hydrogel. The increase in DNA content indicated that there was no significant toxicity from rGO. Additionally, the hydrogels incorporated with rGO were non-toxic to cardiac cells. The spontaneous beating rate was significantly higher for the 5 mg/mL rGO-GelMA samples compared to pure GelMA gels. The rGO-GelMA hydrogels showed higher cell retention.	[166]
GelMA hydrogel	CNTs	The CNTs significantly increased the stiffness of GelMA hydrogels due to their high mechanical properties. Electrical conductivity was improved with the addition of CNTs to the hydrogel. Furthermore, GelMA hydrogels containing dielectrophoretically aligned CNTs achieved higher conductivity compared to GelMA hydrogels with randomly dispersed CNTs.	GelMA hydrogels containing aligned CNTs demonstrated superior performances in supporting cardiac differentiation in mouse embryoid bodies. Protein and gene expression analyses, as well as embryoid beating, were observed in the study.	[167]
GelMA hydrogel with uniaxial architecture	GNRs	The incorporation of GNRs into GelMA hydrogel did not disrupt the macroporous architecture of the GelMA hydrogel matrix. The addition of GNRs influenced the mechanical elasticity, improving the storage modulus, and the electrical conductivity of GelMA-GNR hydrogels, resulting in lower impedance compared to pure GelMA constructs.	The cardiac tissues formed with GelMA-GNR hydrogels exhibited highly dense and uniaxially aligned architectures. The tissues formed in both GelMA and GelMA-GNR constructs demonstrated spontaneous contractility from day 4 to 7 of culture with cells. However, only the electrically conductive GelMA-GNR cardiac tissues showed a consistent response in changing the beating rate as a result of stimulation.	[168]
GelMA Hydrogel	CNTs (single-walled)	CNT/GelMA hydrogels exhibited a well-developed CNT network distributed on the surface of the pore walls. The incorporation of CNTs resulted in an increase in the elastic modulus and Young's compression. It was observed that the conductivity of the CNT-containing hydrogels was significantly higher.	CNTs accelerated the assembly and formation of intercalated discs in the studied cardiac tissues. Additionally, the FAK and RhoA signaling pathways mediated by integrin β1 played a crucial role during this process.	[169]
GelMA Hydrogel + Alginate (bioink)	GNRs	The incorporation of G-GNRs increased the Young's modulus of the hydrogel after	The G-GNR hydrogel (GelMA/GNRs) provided a beneficial microenvironment for	[121]

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Table 2 (*continued*)

Material	Used nanomaterial	Physical Response	Biological response	References
		crosslinking. G-GNRs improved electrical propagation between cardiac cells and enhanced their functional improvement in the printed cardiac construct.	cardiomyocytes' retention, adhesion, growth, and functioning. The printed material with G-GNR nanocomposite bioink exhibited a higher frequency of synchronized contractions.	
GelMA hydrogels embedded electrically conductive gold nanorods (GNRs) and non-conductive silica nanomaterials (SNPs)	Gold nanorods (GNRs) and non-conductive silica nanomaterials (SNPs)	The incorporation of GNRs in GelMA-GNR hydrogel resulted in electrical resistance reduction. Furthermore, impedance measurements indicated that GelMA-SNP hydrogel constructs exhibited high electrical resistance, suggesting that the GelMA-SNP scaffold has insulating properties.	GelMA-GNR demonstrated significantly higher retention of cardiomyocytes, covering approximately 73.6% of the hydrogel constructs. Similarly, GelMA-SNP hydrogel, despite being non-conductive, also exhibited a higher retention rate compared to pristine GelMA. Both GelMA-SNP and GelMA-GNR hydrogels exhibited an improved beating capture rate at frequencies of 3 and 4 Hz.	[170]
GelMA hydrogel	Au/SiO2 hybrid NPs	The SiO2 NRs were coated by Au NPs to produce the hybrid NPs. The incorporation of these NPs improved the mechanical strength and conductivity when compared to GelMA hydrogels.	GelMA-Au/SiO2NPs hydrogels enhanced the biocompatibility and increased H9C2 cardiac cell attachment, with uniformly aligned cell proliferation. In addition, it exhibited controlled swelling and degradation behavior.	[171]
GelMA hybrid hydrogels	CNTs, GO, and rGO	CNT-GelMA hydrogels showed a local elastic modulus of ~16.6 kPa, closer to the physiological stiffness of native neonatal rat cardiac tissue. Also, rGO-GelMA displayed higher levels of FBS adsorption with an ~1.8-fold enhancement compared to pure GelMA.	The hybrid hydrogels, CNT-GelMA and rGO-GelMA, demonstrated favorable characteristics in terms of cardiomyocyte morphology and exhibited a high expression of cardiac markers while preserving cell viability.	[172]
GelMA hydrogel	Gold nanowires (AuNWs)	The addition of AuNWs improved the electrical and mechanical properties of the hydrogel. The Young's modulus of the scaffolds increased with the increase in the concentration of AuNWs, and there was a decrease in swelling capacity with the increase in the concentration of AuNWs.	The addition of AuNWs to the hydrogels did not affect cell viability and showed no cytotoxicity. Compact and densely populated cardiomyocytes with homogeneous distribution were observed in the hybrid hydrogels compared to GelMA hydrogels alone. There was improved cell adhesion with an increase in AuNW concentration up to 0.2 mg/mL, along with high cell viability. Additionally, the presence of AuNWs promoted synchronous beating and a faster spontaneous beating rate.	[107]
GelMA Hydrogel	GO with Polydopamine Reduction (PDA-rGO)	PDA-rGO influenced the electrical conductivity of GelMA hydrogel. The Young's modulus of the hydrogels increased with the concentration of PDA-rGO (0-1 mg/mL).	A higher cell count and uniform distribution were observed in the hydrogels with the nanomaterial. There was an enhanced ability for cell adhesion. Additionally, the beating behavior was accompanied by significantly increased expression of two heart-related proteins and a faster kinetics of Ca_2.	[173]
Conductive microneedle patch GelMA Hydrogel-based	CNTs	GelMA hydrogel integrated with CNTs exhibited an enhanced mechanical stiffness, accompanied by a decrease in elasticity. The incorporation of CNTs into the microneedle patches increased flexibility and conductivity.	The cardiac patch induced the alignment of cardiomyocytes, promoting their synchronous activity. These results also demonstrated adhesion and successful release of the encapsulated proteinic drugs. Furthermore, M-mode echocardiography tests revealed an increase in infarct wall thickness and an improvement in cardiac function.	[174]
GelMA hydrogel and oxidized dextran (ODEX) (GelMA-O)	GO with dopamine reduction	The GelMA-O/rGO hydrogel with 0.5 mg/mL of rGO exhibited an electrical conductivity range similar to that of biological cardiac tissue.	In *in vivo* experiments, the association of the GelMA-O5/rGO hybrid hydrogel with umbilical cord-derived mesenchymal stem cells (UCMSCs) showed positive results. It resulted in a reduction in infarct size and cardiac fibrosis, along with an increase in ventricular ejection fraction. It also positively regulated gene expression and ultimately improved cardiac function after MI.	[148]

[177]), and are being exploited in carbon-derived materials, such as CNTs [178] and graphene [179,180]. However, to date, materials, such as gold and carbon derivatives (CNTs and reduced graphene), are the focus of studies for the production of cardiac tissues associated with GelMA hydrogels, but there are clear opportunities for the development of other nanomaterials with conductive capacity.

The association of GelMA to nanocomposites has shown significant growth in cardiac tissue engineering, due to studies that show the wide variety of uses offered by these materials [181,182]. Their electro-conductivity and the potential to produce nanometric structures make nanocomposites a tool to study cardiac tissues with the objective of regenerating contraction and cell communication [12,162,168,183].

The electrical conductivity of metallic nanocomposites [14,154] makes these materials attractive for use in cardiac tissue engineering with the possibility of assisting in the recovery of cardiac function. Considering the advantages of implementing these materials, several studies have been conducted with the aim of investigating the implementation of different types of nanocomposites for the treatment of CVDs. To promote electrical conductivity to CMs, the biomaterials produced have to present a similar conductivity as the native tissue that typically shows 1 mS/cm [184].

Nanomaterials such as CNTs [169,185], gold nanorods and nanowires [12,121,168], and graphene oxide [148,172,173], are materials that have attractive properties for tissue engineering, high contact surface, chemical stability and the ability to bind to biological molecules without changing their properties [14].

In addition to these characteristics, these materials need to be able to modulate conductivity and improve the stiffness and porosity of the material, bringing more functionality to the cardiac tissue [186]. For example, CNTs and graphene nanomaterials have anisotropic characteristics [180,187]. These materials, in addition to their mechanical and conductive properties [187,188], can promote cell alignment in a single direction, improving conductivity and contractility, favoring their use in cardiac tissues because the heart has an anisotropic nature, and a myocardial electrical microenvironment that enables directional electrical signal propagation.

The behavior of these scaffolds with conductive properties, and how they act by regulating the functionality of cardiac cells, has been studied. It is known that these materials act favoring the adhesion, proliferation and expression of proteins due to the transfer of ions at the site and displacement through the cell membrane. This occurs because there is the formation of electric fields produced by charges within the biomaterial [107,189]. Nanoparticles can act by connecting cells and promoting functional tissue formation, because their nanometric structure increases the surface area, favoring this connection [170,183].

Nanomaterials are added to structures containing cells, with the aim of maintaining the function of primary cardiomyocytes *in vitro* and increase the cell survival by optimizing their functioning [187]. In addition, nanomaterials regulate the differentiation of stem cells into cardiomyocytes, and produce a viable structure for the projection of cardiac tissues, because of their conductivity and mechanical strength. Nanomaterials act similarly to connexin-43 protein (Cx-43), which is synthesized in the plasmatic membrane of cardiomyocytes, in which this protein plays an important role. Cx-43 promotes the formation of intracellular channels with neighboring cardiomyocytes through cytoplasmic components, thus acting directly in the transfer of signaling molecules and ions through cell membranes [14].

Thus, nanomaterials will help in cell sensitization and contribute to synchronous ventricular contraction [190]. In the production of cardiac tissues, the materials should therefore allow cardiomyocytes to develop a mature contractile phenotype (intrinsic functional and structural characteristics of cells) and communicate with adjacent cells (Fig. 5) [191].

6.1. GelMA/gold nanoparticles

Gold nanoparticles (AuNPs) have emerged as one of the most extensively investigated and utilized nanomaterials, primarily owing to their unique attributes. These include easy preparation methods, tunable size and distribution, excellent biocompatibility, adaptability for surface modifications, and the distinct phenomenon of surface plasmon resonance (SPR). SPR arises from the resonant interaction between the nanoparticles and light of specific frequencies, further amplifying their versatility and potential for a wide range of applications. [191]. In addition to being utilized in nanoparticle form, gold has found extensive applications in various formats, such as gold nanorods (GNRs) and gold nanowires (GNWs).

These diverse forms of gold nanomaterials have demonstrated their immense potential across multiple fields, including imaging, diagnostics, tissue engineering, and regenerative medicine. Gold's versatility and unique properties make it a valuable resource for advancing research and innovation in these domains [192]. The addition of gold

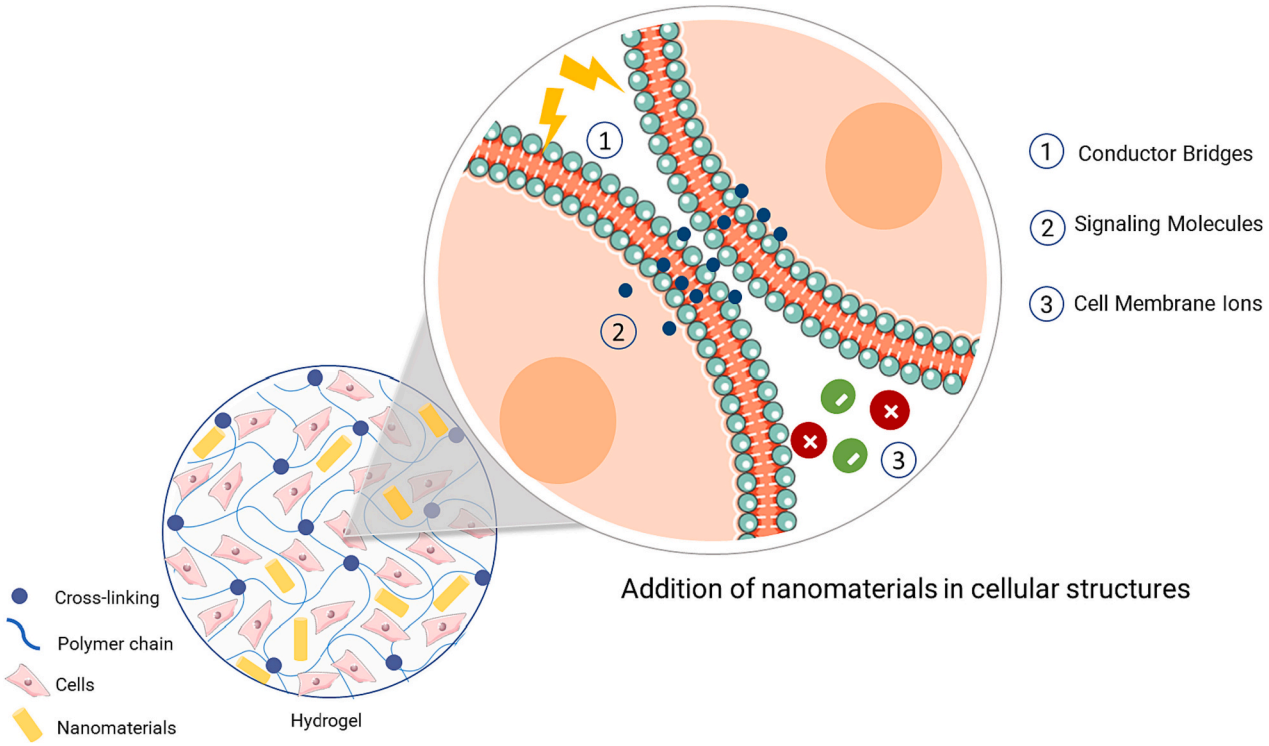

Fig. 5. A schematic example showing enhanced intracellular communications with the addition of nanomaterials in the hydrogel that stimulates communications with adjacent cells in the material. Therefore, producing conductive bridges and exchanging signaling molecules and ions is possible.

nanoparticles (AuNPs) in cardiac constructs favors the creation of conduction bridges, connecting the matrix cardiac cells with nearby cells, promoting electrical propagation in the produced tissue and bringing functionality to the cells [121].

The production of AuNPs can influence their size and morphology, thus one of the main approaches to produce these NPs is through the chemical synthesis of metal ions in solution. In this synthesis, the reduction of Au (III) (HAuCl4) to Au(0) atoms will occur through chemical and stabilizing agents, such as sodium hydroxide (NaOH), sodium borohydride (NaBH4), and others. In addition to chemical methods, the production of AuNPs by physical methodologies is based on the use of a reducing agent without the need for solutions that can generate contamination, such as ion implantation, γ-radiation, optical lithography, microwave, ultrasound, ultraviolet irradiation, among others. Finally, biological synthesis is performed using intracellular or extracellular extracts of microorganisms or plant extracts [193,194].

Studies seek the addition of gold in GelMA biomaterials to improve the characteristics of the hydrogel and enhance its activity. Gold nanoparticles can increase the conductivity of GelMA, improving electrical signal propagation upon electrical stimulation, thus facilitating cell differentiation even in the absence of external electrical stimulation [122] In addition, AuNPs improve cell adhesion and organization due to increased expression of proteins and cardiac enzymes, in addition to the nanometric structure that increases the surface area and adsorption of proteins in place favoring cellular retention, cell-cell coupling, and protein expression [170].

Remarkably, the incorporation of AuNPs into GelMA in the appropriate concentration does not compromise the mechanical rigidity of the material, allowing cells to maintain their regular morphology and engage in their intended activities. These compelling results underscore the immense potential of GelMA-AuNPs bioinks in tissue engineering applications, highlighting their promising role in advancing the field, especially for tissues with high muscle contraction [121,122].

In the study by Navaei et al. (2016) [165], gold nanorods (GNRs) were used in order to promote electrical conductivity of the material. GNRs were synthesized by using a seed-mediated method. For the production of the GNRs, the HAuCl4 solution was mixed with the CTAB (cetyltrimethylammonium bromide) solution; after a color change, it was possible to notice the formation of the NPs. These NPs that were produced are called seed solution and were mixed with a growth solution. An aliquot of the seed solution was then poured into the growth solution and the color of the solution turned reddish brown confirming the formation of GNRs [165].

The incorporation of these GNRs improved the material's stiffness and did not influence physical characteristics, such as porosity. Furthermore, the hydrogel showed a more homogeneous distribution of cardiomyocytes in the hydrogel with GNRs, high adherence, and cell viability due to facilitated cell-cell signaling and electrical signal propagation, as confirmed by the expression of Cx43 communicating junctions and synchronized calcium signaling among cardiomyocytes exposed to high concentrations of GNRs (1.5 mg/mL). Regarding conductivity, the material studied with 1 and 1.5 mg/mL of GNRs showed a significantly higher beat frequency compared to the pure GelMA hydrogel [165].

Similar behavior was seen in the study by Zhu et al. (2017) [121]. In this study, GNRs were applied in a bioink composed of a mixture of GelMA hydrogel with incorporated G-GNRs (GelMA-coated GNRs) and alginate prepolymer solutions for 3D bioprinting. The GNRs were produced with a CTAB coating through a methodology using a seed-mediated surfactant, in which CTAB serves as a compacted bilayer and cationic surfactant. These coated NRs were centrifuged repeatedly to remove excess of CTAB in the stock solution. Subsequently, GelMA molecules were coated onto the surface of the CTAB bilayer under ultrasound and used to reduce the toxicity of CTAB. Previously, G-GNR/GelMA prepolymer solutions were prepared by incorporating G-GNRs at various concentrations (from 0 to 0.5 mg/mL) into a 7% GelMA prepolymer solution [121].

The addition of G-GNRs contributed to the creation of conductive bridges connecting adjacent cells and promoting electrical propagation in the CMs. In the assays with the G-GNR/GelMA hydrogel, a high expression of Cx-43 protein was reported when compared to the pure GelMA hydrogel. A similar result was also described in the materials printed with G-GNRs. For the CMs in the hydrogel, a synchronous beating was shown, and the constructs printed with the nanocomposite showed a higher synchronized contractile frequency when compared to the materials without G-GNRs. Thus, in this study, it was possible to rapidly deposit the fibers loaded with cells at high resolution, decreasing the shear stress on the encapsulated cells. In addition, GelMA was incorporated with GNRs bonded to the electrically resistant pore walls of the polymers, which improved cell-to-cell coupling to allow synchronized shrinkage of the bioprinted constructs [121].

Another study in evidence, using nanomaterials based on gold and GelMA, was done by Li et al. (2020) [12]. In this study, hybrid GelMA hydrogels were associated with gold nanowires (AuNWs) for constructing cardiac tissues *in vitro*. The authors described that AuNWs have rarely been used in the tissue engineering field due to their limited solubility in water. So, to produce the AuNWs, the first synthesis of AuNWs was dispersed in oil followed by a methodology performed with water-dispersion, to form AuNWs able to travel and transmit electrical signals influencing cell contraction through high aspect ratio and high purity level [12].

In the first step, the solution of HAuCl4 was dissolved in a solution of oleylamine and hexane and the triisopropylsilane was used to initiate the reduction process. The AuNWs suspension was purified by precipitation and redispersed in dichloromethane. In the second step, the 3-mercaptopropionic acid (MPA) solution was added to the solution containing AuNWs dispersed in oil. The AuNWs were transferred from the organic phase to the water phase, so the aqueous solution was subjected to sonication and concentrated in the AuNWs [12].

As a result, a hydrogel was obtained to promote the electrical and mechanical properties for application in cardiac tissues *in vitro*. The characterizations performed on the material confirmed the obtained GelMA hydrogels with AuNWs seeded with cardiomyocytes with excellent properties. The cardiomyocytes seeded on GelMA hydrogels were beating randomly and unsynchronized, whereas cardiomyocytes showed strong spontaneous synchronous beating activity in all AuNWs-hybrid hydrogel groups. The cells incorporated in the GelMA hydrogels with AuNWs showed high viability, maturation, and were beating synchronously, besides expressing specific cardiac proteins as Cx 43 [12].

6.2. GelMA/graphene biomaterials

The development of carbon-based biomaterials has favored research into nanomaterials, such as graphene and its derivatives, graphene oxide (GO) and reduced graphene oxide (rGO), which have emerged as a new strategywith respect to biomedical applications, such as regenerative medicine, tissue engineering and controlled drug delivery. Graphene-based nanomaterials draw attention due to their simple physical and chemical properties, low cost, and stand out due to their conductivity characteristics [172,195].

Graphene, as the basic structure of graphite, is made up of a single layer of carbon atoms and has hydrophobic characteristics, which make this biomaterial less stable and susceptible to agglomeration [173]. Oxidation of graphite results in the synthesis of GO, which is the graphene derivative most used in studies related to tissue engineering, because of the functional groups of oxygen and the high aromatic interface allow the GO interaction with DNA, peptides, or proteins through physisorption or chemical binding [195].

On the other hand, GO has a suppressed thermal conductivity compared to graphene, however it can be reduced through chemical, thermal, solvothermal, electrochemical, microwave or photoreduction techniques and thus produces rGO. In this process, oxygen functional

groups are eliminated from GO, improving electrical conductivity and increasing interlayer distance and functionality [196]. rGO can engage in electrostatic interactions that occur through π-π stacking between sheets and proteins. It can also participate in hydrophobic interactions with the hydrophobic regions of proteins [166,197].

For the production of GO, graphite is oxidized in an acidic medium following the methodology of Hummers and Offermann. In this methodology, the graphite is dispersed in oxidizing and stabilizing media, after this process the material is oxidized again in a deep way and hydrolyzed to obtain a brown suspension that signals the total exfoliation of the graphite oxide. The residual oxidants are reduced with H2O2 and washed several times with HCl and water. In addition to chemical syntheses using various substances, electrochemical synthesis represents an approach to GO synthesis that is more environmentally friendly than chemical production and is currently widely studied [198,199].

For the reduction of GO, several methodologies, such as chemical reduction, thermal reduction, photo-reduction, and microwave reduction can be used. Among them, chemical reduction is the most used technique for the synthesis of rGO, this is an easy method of production and application, in addition to being more cost-effective [199]. Ascorbic acid is one of the most studied reducing agents for the synthesis of rGO, due to its low cost and the fact that it is ecological and highly efficient. In addition, it is capable of forming a stable dispersion with good biocompatibility [200].

The UV-induced cross-linking efficiency of GelMA was decreased with the incorporation of GO, thus GO reduced with ascorbic acid improves electrical conductivity and biocompatibility, and GelMA acts as a biocompatible substrate to provide active chemical groups. This composition provides a natural cellular microenvironment for cardiomyocyte culture and maintains proper cell adhesion, so that it achieves optimal electrical conductivities for cardiac applications in tissue engineering [201].

Shin et al. (2013) [185] introduced the graphene oxide (GO) associated with GelMA hydrogels. In this study, fibroblast cells were incorporated with graphene, and the cellular responses were investigated in a 3D microenvironment. The hybrid hydrogels (GO-GelMA) were able to support cell spreading and alignment, as well as improved viability and proliferation. In addition, mechanical properties were improved, with GO-GelMA hydrogels, GO concentrations (0–2.0 mg/mL), as well as improved electrical conductivity, opening new avenues for 3D tissue engineering for the treatment of myocardial infarction.

Following the application of GO, in Paul et al. (2014) [163], hydrogels based on GO/GelMA nanocomposites were studied as an injectable gene delivery agent to enhance myocardial tissue repair mechanisms. In their study, GelMA was impregnated with GO nanosheets (fGO) forming a nanocomplex of GO and pro-angiogenic vascular endothelial growth factor-165 (VEGF) gene (fGOVEGF). The addition of fGO significantly increased the viscosity of the hydrogel. This was attributed to the physical interaction of the surface-functionalized fGO nanosheets with GelMA. Furthermore, with the addition of fGO to GelMA, the mechanical strength of the hydrogel was enhanced. In the *in vivo* study, it was observed that injectable fGOVEGF hydrogels induced positive effects on tissue revascularization at the injury site and improved contractile performance, as corroborated by vasculogenesis [163].

In the study by Shin et al. (2016) [166], reduced GO with ascorbic acid was used making the process biocompatible and less cytotoxic. The authors showed that the incorporation of GelMA with rGO for the construction of cardiac tissue has the potential for the electrical conductivity and mechanical properties of the material so that it makes a more natural microenvironment for the cardiomyocytes. In addition, it showed better cell viability, proliferation, and maturation. Furthermore, cardiomyocytes showed stronger contractility and a faster spontaneous beating rate in the rGO-GelMA hydrogel sheets compared to the pure GelMA hydrogels, as well as in the GO-GelMA hydrogel sheets with similar mechanical properties and particle concentrations [166].

In another study, conducted by Li et al. (2021) [173], the GO was reduced with polydopamine (PDA-rGO) and was prepared and incorporated into gelatin methacrylate hydrogels (GelMA) to construct cardiac microtissues. For this, a solution of GO and dopamine hydrochloride was prepared and allowed to react for 60 min. Next, a Tris-HCl solution was added to the mixture. The reaction was carried out resulting in the formation of a dark suspension. Finally, to form GelMA-PDA-rGO hybrid hydrogels, the lyophilized GelMA was dissolved in PBS and the photoinitiator LAP was added at 50 °C. Finally, different concentrations of PDA-rGO were added to the solution for further analysis [173].

In vitro tests with CMs showed greater cytocompatibility compared to cells cultured in purine GelMA hydrogels, shown by rates of higher cell survival and upregulation of cardiac proteins. Also, PDA-rGO dispersion showed great stability and prevented aggregation for several months, which ccould be attributed to the presence of hydrophilic hydroxyl groups in PDA. [173].

In a recent study by Zhu et al. (2022) [148], a GelMA-based hydrogel system was proposed as a delivery vehicle for umbilical cord mesenchymal stem cells (UCMSCs). The hydrogel system combined with oxidized dextran (ODEX) was introduced to increase the external energy dissipation and reduce the degradation rate of the hydrogel. ODEX was synthesized by adding sodium periodate to initiate oxidation and dialyzing the solution to obtain ODEX polymer [148].

Furthermore, the study used dopamine as a reductant for GO to improve its properties, by stirring GO and dopamine hydrochloride under alkaline conditions [148]. GelMA-O5/rGO (ODEX 50 mg/mL) exhibited the highest compression modulus among the three concentrations tested. However, the addition of ODEX and rGO resulted in a reduced swelling rate of the hydrogel, potentially due to the formation of a more compact structure with increased cross-link density [148].

The results showed that UCMSCs adhered and spread on the hydrogel surface, maintaining normal cell morphology and enhancing cellular differentiation efficiency. GelMA-O5/rGO promoted an up-regulation of cardiac troponin I (cTnI) and Cx-43, both associated with the proper function and regulation of healthy cardiac tissue [148].

Thus, hybrid hydrogel systems can be formed by combining GelMA with nanoparticles, such as carbon nanotubes and graphene oxide, and other polymers to form networks with expected properties and characteristics for specific biological applications [202].

6.3. GelMA/carbon nanotubes

Carbon nanotubes (CNTs) are one of the nanoscale allotropic forms of carbon that form a cylinder-like structure with a large surface area [203]. The CNTs can be made of two types, depending on the number of graphite layers. Single-walled carbon nanotubes (SWCNTs) have a cylindrical structure in a single tube-like layer. Multi-walled carbon nanotubes (MWCNTs), on the other hand, have a structure with several concentric coaxial sheets of carbon [204].

CNTs can have different properties in terms of length, size, and charge [205]. In addition, parallel-aligned CNTs can promote effective interaction between excitable cells and induce directional cell growth, while randomly dispersed CNTs are more likely to create agglomerates [167,174]. SWCNTs can present more reliable conductive properties, but despite their advantages, the use of SWCNTs can present complications due to their nanometer scale being able to penetrate the cell membrane and disrupt intracellular processes [206]. Hence, MWCNTs are most frequently used in cardiac tissue engineering with diameters ranging from 10 to 200 nm [162,164,167,172,174].

CNTs and GelMA have been applied to improve the quality of modified cardiac tissue for the purpose of regenerative therapies because they support the growth and functioning of cardiomyocytes [207]. In addition, they benefit cell performance by improving protein expression, aiding in cell maturation, and improving cell alignment and communication [208]. The use of CNT/GelMA hydrogel has the

potential to impact cell phenotype and improve cell adhesion. This creates an advantageous ECM-like environment that fosters enhanced intercellular junction and cell-cell coupling, leading to enhanced cell conductivity and, therefore, cardiomyocyte maturation. Additionally, cardiac tissues based on CNTs/GelMA hydrogels showed better functionality because their intracellular Ca^{2+} transients significantly increase, a characteristic that favors the contraction cycle [169].

Since effective production methods for CNTs are crucial for their proper utilization, chemical vapor deposition method has been proposed and is currently one of the most used techniques, as it allows precise control of CNT formation, producing CNTs in different sizes, structures, and properties by providing energy to a carbon source for the formation of carbon atoms that generate CNTs [209]. To improve the functionality of CNTs for various studies, chemical or coating functionalization are performed [164,167]. In addition, some studies perform the ordered arrangement of CNTs to facilitate cell-cell interaction and directional cell growth [174,210].

Applying CNTs in cell studies, Shin et al. (2011) [181] showed that the number of cells encapsulated in CNT-GelMA increased more than the double from 24 to 48 h, proliferating more rapidly than those in the GelMA-only controls. Thus, GelMA showed a strong affinity for MWCNTs, in addition to preserving the functional structure, which makes possible a higher rate of cell proliferation due to stronger adhesion compared to GelMA alone.

According to the study by Shin et al. (2013) [162], the incorporation of multi-walled CNTs into GelMA was prepared by the addition of carboxylic acid functionalized CNTs to the GelMA solution, and sonicated to obtain a solution with CNTs coated in GelMA. Hydrogel-based thin films (CNT-GelMA) were produced by UV irradiation. The pre-polymer solution with the CNTs was added between two glass slides and photopolymerized to build up the film [162].

The result improved the hydrogel's mechanical integrity and electrophysiological functions so that cardiac tissues cultured in CNT-GelMA obtained stable beats, capable of conducting contractile behaviors and protective activity against cardiotoxic and cardioinhibitory substances. The percentage of retention, viability, and cell alignment index was influenced by the concentration of CNTs. This occurred because CNTs formed electrically conductive nanofibers that improved the mechanical strength of the hydrogel, thus promoting adhesion and maturation of cardiac cells and improving cell-cell electrical coupling. Moreover, CNTs 3 mg/mL led to tissues with optimal electrophysiological functions, while 5 mg/mL showed the maximum protective effect [162].

In the Ahadian et al. (2016) [167] study, CNTs hydrogels aligned with GelMA were developed to support cardiac differentiation of mouse embryoid bodies (EBs). CNTs showed better performance in generating functional and contractile skeletal muscle myofibers. For this purpose, pure GelMA and GelMA hydrogels containing dielectrophoretically aligned and random CNTs at different CNT concentrations (0.25; 0.5 and 1 mg/mL) were prepared. The hydrogels containing aligned CNTs obtained a higher conductivity compared to the randomly aligned CNTs, this is because the aligned CNT network favors a direct propagation of the electric current more easily. The conductivity measurements at an applied voltage of 5 V for GelMA hydrogels containing 0.5 mg/mL aligned and random CNTs were 24.2 × 10–7 mA and 1.36 × 10–7 mA, respectively [167].

In the *in vitro* studies, the aligned CNTs showed better support for the cardiac differentiation of EBs, due to the more effective targeting and control of cell differentiation that the aligned CNTs provide. In addition, when analyzing proteins and genes, EBs added with aligned CNTs had a higher activity in the beat, and electrical stimulation was responsible for further increasing the beats of EBs, with greater effects on those containing aligned CNTs in the hydrogel. Thus, it was possible to obtain GelMA and CNT hydrogels aligned with excellent results for future application in regenerative medicine and cell therapy [167].

Sun et al. (2017) [169] demonstrated that SWCNTs-based hydrogels support cardiomyocytes in a way that increase cell adhesion and maturation. Furthermore, it was shown that hydrogels with CNT concentrations ranging from 50 to 200 ppm have great mechanical resistance and higher conductivity, in comparison to GelMA hydrogels. The electrically conductive (Ω^{-1}) GelMA hydrogel a conductivity of 4.73 × 10–12, while for CNTs hydrogels, the conductivity were as follows: 50 ppm = 1.90×10^{-11}, 100 ppm = 1.72×10^{-9}, 150 ppm = 1.85×10^{-7}, and 200 ppm = 1.77×10^{-6} as shown in the complementary material. These numbers highlight the higher electrical conductivity with the addition of CNTs [169].

The improved conductivity feature of the embedded hydrogels provides a favorable microenvironment for intercellular junction and cell coupling. The CNTs incorporated into hydrogels demonstrated the ability to regulate electrical conductivity by activating downstream signaling kinases FAK (focal adhesion kinase) and Rho, which play a crucial role in intercalated disc formation. Inhibition of FAK leads to impaired gap junction protein Cx-43, demonstrating the ability of CNTs to create a favorable environment for myocardial cell function. In the *in vitro* analysis, there was a decrease in the degradation rate of the GelMA hydrogels, so this incorporation resulted in the improvement of the morphological, mechanical, and electrical properties of the hydrogels [169].

Hence, it is postulated that CNT-GelMA constructs exhibit enhanced electrical conductivity and superior mechanical strength compared to traditional GelMA hydrogels. This notable combination of properties positions CNT-GelMA as a highly promising material for myocardial tissue engineering, presenting an exciting avenue for advancing tissue quality and for optimizing its applicability in cardiac regenerative therapies.

7. Further applications of GelMA and other nanomaterials for understudied CVDs

GelMA and its versatility are also associated with various areas of regenerative medicine and cardiac tissue engineering. Studies have already explored this material as bioinks for 3D bioprinting of cardiac tissues and organs [66,211], heart-on-chip for drug screening [212,213], heart valves [146,214] and vascularized tissues [15,215].

Nanotechnology has become an ally in tissue engineering. These nanomaterials are shaped on the nanometric scale, which improves the surface area, mechanical and electrical resistance, and evenantimicrobial and antiseptic properties of the materials produced [16]. In addition, there are other types of nanomaterials that have not yet been explored in the cardiac context, but have already been applied in tissue engineering for various purposes, such as silica-based nanoparticles [216], hydroxyapatite [217], silver nanoparticles for microbial and healing action [218], and solid lipid nanoparticles [219]. These materials are promising and could be studied in the cardiac area, both to help the delivery of genes and growth factors, and to make the materials more resistant, for applications in tissue bioprinting, vascular tissue production and others.

GelMA polymer exhibits gelling properties and can be extruded, which are ideal and essential properties for 3D printing. Therefore, when at an appropriate temperature, GelMA can be deposited and form the appropriate structure [211]. In the study by Hu et al. (2022) [220], cardiomyoblasts were mixed in GelMA bioinks and printed using an extrusion-based 3D bioprinter. The GelMA scaffolds were successfully manufactured, as their structural integrity was maintained for more than 30 days, adequate time to obtain the consistency of the cell growth space, which provides a promising material for *in vivo* studies.

In the study by Lee et al. (2020) [15], GelMA and recombinant human tropoelastin were used to make a bioink with biocompatibility and mechanical characteristics suitable for 3D printing. The results demonstrated that the bioink has the potential to print functional cardiac tissues in 3D so that it allowed a high-resolution impression and cell viability, so there was cell proliferation without modifying the phenotype of the cells, which presented a heartbeat similar to cardiac cells, an

essential function, and was shown to be biodegradable *in vivo*.

Other studies focus on the development of *in vitro* cardiac tissue using microfluidic technology to create a controllable microenvironment for cell development and maintenance. These heart-on-a-chip systems will simulate cardiac tissue *in vitro* and be applied to model heart disease and assist in drug screening. This method reduces the number of studies using animals and is more reliable than the 2D models currently used [221,222].

In the study conducted by Chen et al. (2013) [212], with the aim of creating an "organ-on-a-chip" model, a membrane microfluidic platform was developed. This platform consists of two channels, one upper and one lower, which allow cells to pass through where the hydrogel has been introduced, serving as a matrix for the transport of cells within this microenvironment. Thus, the platform seeks to replicate the interactions with the aim of investigating the impact of valvular endothelial cells on valvular interstitial cells for the development of alternative therapies for valvular diseases.

Despite the versatility of applying GelMA to cardiac studies, in order to recreate these complex structures, it is necessary to find ways of integrating the tissues produced with a vascularization network, which is one of the biggest challenges in these studies [223]. Thus, one of the most discussed topics in the cardiac area is the possibility of creating vascularized tissues. Vascularization is necessary to increase the survival of SMCs and cardiac function [224].

Currently, tissue engineering aims to produce vessels similar to those *in vivo*, capable of being biocompatible, withstanding changes in systemic pressure and also supporting physiological flow. This can occur through the release of angiogenic factors to induce the growth of a vascular network in the tissue produced *in vivo*, or produced directly by technologies, such as 3D printing [225].

In the study by Benton et al. (2009) [144], GelMA was associated with aortic valve interstitial cells (VICs), an important cell line for heart valve tissue engineering. In this study, the transforming growth factor-β1 was also encapsulated with the cells. As a result, it was possible to obtain photoreticulable hydrogels with cells capable of achieving a native morphology even in the control group without the presence of the TGF-β1 factor. In addition, it was possible to visualize the increased expression of α-smooth muscle actin and collagen-1, indicating a differentiation of fibroblasts into active myofibroblasts.

Still aiming at the production of heart valves, the study by Ravishankar et al. (2020) [146] applied a hybrid hydrogel of GelMA and glycosaminoglycan (GAG) mixture containing sodium hyaluronate and chondroitin sulfate, incorporating anisotropic polycaprolactone fibers, produced by centrifugal jet spinning (CJS) technique. In addition, porcine aortic valve interstitial cells (VICs) were incorporated into the scaffold. As a result, it was possible to obtain a favorable environment for the cells, with high viability expressing positive activation and proliferation markers. The fibers were able to reinforce the material, and its mechanical behavior was similar to that of native porcine aortic valves. Also, Su et al. (2018) [224] developed biomimetic microvessels specifically engineered to serve as transport conduits, enabling the stem cells within the patch to access essential nutrients from the surrounding tissue. The coating of the biomimetic microvessels was achieved by utilizing an interpenetrating network of PEG/GelMA hydrogel. Furthermore, they demonstrated being able to induce angiogenesis in the ischemic region resulting from AMI by integrating engineered blood vessels, thereby promoting both cardiomyocyte proliferation and neovascularization following heart failure.

With a view to clinical application, the engineered tissues must present specific cardiac cells, with optimal phenotypic and immunological characteristics. The tissue must be in a matrix similar to the characteristics of physiological cardiac tissue. It must also be of a size and thickness suitable for clinical application. It must be able to connect electromechanically and vascularly with the patient's heart [226].

Despite advances in the development of structures, cardiac tissue engineering has a long way to go before it can be clinically applied. Many of the tissues produced are not completely suitable for routine clinical application, but there have already been therapeutic advances with excellent results [86]. The current difficulties in terms of maintaining structures are due to the fact that hydrogels still need to be integrated into a vascular network, in addition to the need to assess cell rearrangement within the hydrogel, which is often different from the native tissue [227].

Based on these findings, GelMA and nanomaterials are promising for cardiac studies, the development of structures and new techniques for producing new materials. In this way, they can help in the treatment of CVDs improving quality of life of patients. In addition, the development of these structures can help with cardiac deficiencies, such as transplants. However, advanced techniques to overcome these deficiencies are still needed, and nanomaterials have become a useful tool for adding mechanical strength, cell stability and electrical conductivity to the tissues produced.

8. Conclusions

This review focuses on the latest advances on the use of GelMA as an important biomaterial to be associated with nanomaterials for the development of new treatment options for CVDs. The increasingly interest on the exploitation of bioprinting technologies to build cardiac tissue opens new opportunities to further exploit GelMA in the repair of cardiac tissues. Combined with nanomaterials, such as gold, carbon nanotubes and graphene, the obtained composites and scaffolds have been studied as conductive agents, acting on the mechanical structure, in order to amplify cellular communication. Besides, GelMA 3D printing composites are biocompatible and offer the required mechanical properties that are instrumental for the construct of a suitable environment for the proliferation of cardiomyocytes in tissue regeneration, aiming at the treatment of CVDs.

Ethics issues

This work does not raise any ethics issues.

CRediT authorship contribution statement

Erika S. Lisboa: Writing – original draft, Conceptualization. **Carine Serafim:** Writing – original draft, Methodology. **Wanessa Santana:** Writing – original draft, Methodology. **Victoria L.S. dos Santos:** Validation, Investigation. **Ricardo L.C. de Albuquerque-Junior:** Validation, Resources, Investigation. **Marco V. Chaud:** Validation, Resources, Investigation. **Juliana C. Cardoso:** Validation, Supervision, Formal analysis. **Sona Jain:** Visualization, Supervision, Formal analysis. **Patrícia Severino:** Writing – original draft, Project administration, Conceptualization. **Eliana B. Souto:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

Acknowledgment

The author's acknowledgement Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Sergipe (FAPITEC), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 304590/2022-3, 408377/2022-4). FCT—Fundação para a Ciência e a Tecnologia, I.P. is also acknowledged within the scope of the projects UIDP/04378/2020 and UIDB/04378/

2020 of the Research Unit on Applied Molecular Biosciences—UCIBIO, and LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy—i4HB.

References

[1] Y. Hu, W. Chen, H. Yin, X. Chen, J. Cai, J. Guo, S. Zhou, R. Chai, M. Tang, Super-aligned carbon nanotubes and GelMA hydrogel composite scaffolds promote spiral ganglion neuron growth and orientation, Mater. Today Nano 18 (2022), 100181.

[2] F.D. Fuchs, P.K. Whelton, High blood pressure and cardiovascular disease, Hypertension 75 (2020) 285–292.

[3] M. Vaduganathan, A. Mensah George, V. Turco Justine, V. Fuster, A. Roth Gregory, The global burden of cardiovascular diseases and risk, J. Am. Coll. Cardiol. 80 (2022) 2361–2371.

[4] N. Liu, X. Ye, B. Yao, M. Zhao, P. Wu, G. Liu, D. Zhuang, H. Jiang, X. Chen, Y. He, Advances in 3D bioprinting technology for cardiac tissue engineering and regeneration, Bioact. Mater. 6 (2021) 1388–1401.

[5] R. Dong, P.X. Ma, B. Guo, Conductive biomaterials for muscle tissue engineering, Biomaterials 229 (2020), 119584.

[6] R. Portillo-Lara, A.R. Spencer, B.W. Walker, E. Shirzaei Sani, N. Annabi, Biomimetic cardiovascular platforms for in vitro disease modeling and therapeutic validation, Biomaterials 198 (2019) 78–94.

[7] A.W. Holle, J.L. Young, K.J. Van Vliet, R.D. Kamm, D. Discher, P. Janmey, J. P. Spatz, T. Saif, Cell-extracellular matrix Mechanobiology: forceful tools and emerging needs for basic and translational research, Nano Lett. 18 (2018) 1–8.

[8] B. Zhang, L. Gao, L. Ma, Y. Luo, H. Yang, Z. Cui, 3D bioprinting: a novel avenue for manufacturing tissues and organs, Engineering 5 (2019) 777–794.

[9] G. Jose, K. Shalumon, J.-P. Chen, Natural polymers based hydrogels for cell culture applications, Curr. Med. Chem. 27 (2020) 2734–2776.

[10] T. Alves, J. Souza, L. Silveira Filho, Dense lamellar scaffold as biomimetic materials for reverse engineering of myocardial tissue: preparation, characterization and physiomechanical properties, J. Mater. Sci. Eng. 7 (2018) 1–10.

[11] A.B. Bello, D. Kim, D. Kim, H. Park, S.-H. Lee, Engineering and functionalization of gelatin biomaterials: from cell culture to medical applications, Tissue Eng. Part B Rev. 26 (2020) 164–180.

[12] X.-P. Li, K.-Y. Qu, F. Zhang, H.-N. Jiang, N. Zhang, C. Nihad, C.-M. Liu, K.-H. Wu, X.-W. Wang, N.-P. Huang, High-aspect-ratio water-dispersed gold nanowires incorporated within gelatin methacrylate hydrogels for constructing cardiac tissues in vitro, J. Mater. Chem. B 8 (2020) 7213–7224.

[13] R.K. Kankala, K. Zhu, X.-N. Sun, C.-G. Liu, S.-B. Wang, A.-Z. Chen, Cardiac tissue engineering on the nanoscale, ACS Biomater. Sci. Eng. 4 (2018) 800–818.

[14] K. Ashtari, H. Nazari, H. Ko, P. Tebon, M. Akhshik, M. Akbari, S.N. Alhosseini, M. Mozafari, B. Mehravi, M. Soleimani, Electrically conductive nanomaterials for cardiac tissue engineering, Adv. Drug Deliv. Rev. 144 (2019) 162–179.

[15] S. Lee, E.S. Sani, A.R. Spencer, Y. Guan, A.S. Weiss, N. Annabi, Human-recombinant-elastin-based bioinks for 3D bioprinting of vascularized soft tissues, Adv. Mater. 32 (2020) 2003915.

[16] F. Habibzadeh, S.M. Sadraei, R. Mansoori, N.P. Singh Chauhan, G. Sargazi, Nanomaterials supported by polymers for tissue engineering applications: a review, Heliyon 8 (2022), e12193.

[17] A. Abdal Dayem, S.B. Lee, S.-G. Cho, The impact of metallic nanoparticles on stem cell proliferation and differentiation, Nanomaterials 8 (2018) 761.

[18] A.G. Kurian, R.K. Singh, K.D. Patel, J.-H. Lee, H.-W. Kim, Multifunctional GelMA platforms with nanomaterials for advanced tissue therapeutics, Bioact. Mater. 8 (2022) 267–295.

[19] J. Liang, P. Dijkstra, A. Poot, D. Grijpma, Hybrid hydrogels based on methacrylate-functionalized gelatin (GelMA) and synthetic polymers, Biomed. Mater. Devic. (2022) 1–11.

[20] N.J. van Eck, L. Waltman, Software survey: VOSviewer, a computer program for bibliometric mapping, Scientometrics 84 (2010) 523–538.

[21] D.A. Watkins, W.T. Msemburi, S.J. Pickersgill, Y. Kawakatsu, A. Gheorghe, K. Dain, K.A. Johansson, S. Said, N. Renshaw, M.T. Tolla, P.D. Twea, C. Varghese, K. Chalkidou, M. Ezzati, O.F. Norheim, NCD countdown 2030: efficient pathways and strategic investments to accelerate progress towards the sustainable development goal target 3.4 in low-income and middle-income countries, Lancet 399 (2022) 1266–1278.

[22] S. Kaptoge, L. Pennells, D. De Bacquer, M.T. Cooney, M. Kavousi, G. Stevens, L. M. Riley, S. Savin, T. Khan, S. Altay, World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions, the lancet, Glob. Health 7 (2019) e1332–e1345.

[23] W.H. Organization, Cardiovascular diseases World Health Organization, Cardiovascular diseases. Fact Sheet vol. 317, 2021.

[24] B. Şahin, G. İlgün, Risk factors of deaths related to cardiovascular diseases in World Health Organization (WHO) member countries, Health Soc. Care Commun. 30 (2022) 73–80.

[25] S. Yusuf, P. Joseph, S. Rangarajan, S. Islam, A. Mente, P. Hystad, M. Brauer, V. R. Kutty, R. Gupta, A. Wielgosz, Modifiable risk factors, cardiovascular disease, and mortality in 155 722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study, Lancet 395 (2020) 795–808.

[26] A. Johansson, I. Drake, G. Engström, S. Acosta, Modifiable and non-modifiable risk factors for atherothrombotic ischemic stroke among subjects in the Malmö diet and cancer study, Nutrients 13 (2021) 1952.

[27] E.J. Schaefer, H. Ikezaki, M.R. Diffenderfer, E. Lim, C.-T. Liu, R.C. Hoogeveen, W. Guan, M.Y. Tsai, C.M. Ballantyne, Atherosclerotic cardiovascular disease risk and small dense low-density lipoprotein cholesterol in men, women, African Americans and non-African Americans: The pooling project, Atherosclerosis 367 (2023) 15–23.

[28] A. Kumar, V. Siddharth, S.I. Singh, R. Narang, Cost analysis of treating cardiovascular diseases in a super-specialty hospital, PLoS One 17 (2022), e0262190.

[29] C.C. Huber, X. Wang, H. Wang, Impact of cardiovascular diseases on ischemic stroke outcomes, J. Integr. Neurosci. 21 (2022) 138.

[30] A.S. Khaku, P. Tadi, Cerebrovascular disease, in: StatPearls, Treasure Island (FL) Ineligible Companies. Disclosure: Prasanna Tadi Declares no Relevant Financial Relationships with Ineligible Companies, StatPearls Publishing, 2023 (Copyright © 2023, StatPearls Publishing LLC., StatPearls Publishing).

[31] C.W. Tsao, A.W. Aday, Z.I. Almarzooq, C.A.M. Anderson, P. Arora, C.L. Avery, C. M. Baker-Smith, A.Z. Beaton, A.K. Boehme, A.E. Buxton, Y. Commodore-Mensah, M.S.V. Elkind, K.R. Evenson, C. Eze-Nliam, S. Fugar, G. Generoso, D.G. Heard, S. Hiremath, J.E. Ho, R. Kalani, D.S. Kazi, D. Ko, D.A. Levine, J. Liu, J. Ma, J. W. Magnani, E.D. Michos, M.E. Mussolino, S.D. Navaneethan, N.I. Parikh, R. Poudel, M. Rezk-Hanna, G.A. Roth, N.S. Shah, M.P. St-Onge, E.L. Thacker, S. S. Virani, J.H. Voeks, N.Y. Wang, N.D. Wong, S.S. Wong, K. Yaffe, S.S. Martin, Heart disease and stroke Statistics-2023 update: a report from the American Heart Association, Circulation 147 (2023) e93–e621.

[32] T. Auala, B.G. Zavale, A. Mbakwem, A.O. Mocumbi, Acute Rheumatic Fever and Rheumatic Heart Disease: Highlighting the Role of Group a Streptococcus in the Global Burden of Cardiovascular Disease, Pathogens (Basel, Switzerland) vol. 11, 2022.

[33] C. Dass, A. Kanmanthareddy, Rheumatic Heart Disease, in: StatPearls, Treasure Island (FL) Ineligible Companies. Disclosure: Arun Kanmanthareddy Declares no Relevant Financial Relationships With Ineligible Companies, StatPearls Publishing, 2023 (Copyright © 2023, StatPearls Publishing LLC., StatPearls Publishing).

[34] K. Williams, J. Carson, C. Lo, Genetics of congenital heart disease, Biomolecules 9 (2019).

[35] h.w.w.i.n.-r.f.-s.d.c.-d.-c.a.o.s.N. 2023), (2023).

[36] E. Marijon, M. Mirabel, D.S. Celermajer, X. Jouven, Rheumatic heart disease, Lancet (Lond., Engl.) 379 (2012) 953–964.

[37] R. Sun, M. Liu, L. Lu, Y. Zheng, P. Zhang, Congenital heart disease: causes, diagnosis, symptoms, and treatments, Cell Biochem. Biophys. 72 (2015) 857–860.

[38] G.A. Roth, D. Abate, K.H. Abate, S.M. Abay, C. Abbafati, N. Abbasi, H. Abbastabar, F. Abd-Allah, J. Abdela, A. Abdelalim, Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of disease study 2017, Lancet 392 (2018) 1736–1788.

[39] O.J. Mechanic, M. Gavin, S.A. Grossman, Acute Myocardial Infarction, in: StatPearls, Treasure Island (FL) ineligible Companies. Disclosure: Michael Gavin Declares No Relevant Financial Relationships With Ineligible Companies. Disclosure: Shamai Grossman Declares No relevant Financial Relationships With Ineligible Companies, StatPearls Publishing, 2023 (Copyright © 2023, StatPearls Publishing LLC., StatPearls Publishing).

[40] N. Ojha, A.S. Dhamoon, Myocardial infarction, in: StatPearls, Treasure Island (FL) Ineligible Companies. Disclosure: Amit Dhamoon Declares No Relevant Financial Relationships With Ineligible Companies, StatPearls Publishing, 2023 (Copyright © 2023, StatPearls Publishing LLC., StatPearls).

[41] R.J. Vagnozzi, M. Maillet, M.A. Sargent, H. Khalil, A.K.Z. Johansen, J. A. Schwanekamp, A.J. York, V. Huang, M. Nahrendorf, S. Sadayappan, An acute immune response underlies the benefit of cardiac stem cell therapy, Nature 577 (2020) 405–409.

[42] H. Hashimoto, E.N. Olson, R. Bassel-Duby, Therapeutic approaches for cardiac regeneration and repair, Nat. Rev. Cardiol. 15 (2018) 585–600.

[43] Y. Li, L. Wei, L. Lan, Y. Gao, Q. Zhang, H. Dawit, J. Mao, L. Guo, L. Shen, L. Wang, Conductive biomaterials for cardiac repair: a review, Acta Biomater. 139 (2022) 157–178.

[44] T. Wu, C. Cui, Y. Huang, Y. Liu, C. Fan, X. Han, Y. Yang, Z. Xu, B. Liu, G. Fan, Coadministration of an adhesive conductive hydrogel patch and an injectable hydrogel to treat myocardial infarction, ACS Appl. Mater. Interfaces 12 (2019) 2039–2048.

[45] V. Talman, H. Ruskoaho, Cardiac fibrosis in myocardial infarction-from repair and remodeling to regeneration, Cell Tissue Res. 365 (2016) 563–581.

[46] D. Jenča, V. Melenovský, J. Stehlik, V. Staněk, J. Kettner, J. Kautzner, V. Adámková, P. Wohlfahrt, Heart failure after myocardial infarction: incidence and predictors, ESC Heart Fail. 8 (2021) 222–237.

[47] B. Chapman, A.D. DeVore, R.J. Mentz, M. Metra, Clinical profiles in acute heart failure: an urgent need for a new approach, ESC Heart Fail 6 (2019) 464–474.

[48] E.J. Benjamin, M.J. Blaha, S.E. Chiuve, M. Cushman, S.R. Das, R. Deo, S.D. de Ferranti, J. Floyd, M. Fornage, C. Gillespie, C.R. Isasi, M.C. Jiménez, L.C. Jordan, S.E. Judd, D. Lackland, J.H. Lichtman, L. Lisabeth, S. Liu, C.T. Longenecker, R. H. Mackey, K. Matsushita, D. Mozaffarian, M.E. Mussolino, K. Nasir, R. W. Neumar, L. Palaniappan, D.K. Pandey, R.R. Thiagarajan, M.J. Reeves, M. Ritchey, C.J. Rodriguez, G.A. Roth, W.D. Rosamond, C. Sasson, A. Towfighi, C. W. Tsao, M.B. Turner, S.S. Virani, J.H. Voeks, J.Z. Willey, J.T. Wilkins, J.H. Wu, H.M. Alger, S.S. Wong, P. Muntner, Heart disease and stroke Statistics-2017

update: a report from the American Heart Association, Circulation 135 (2017) e146–e603.
[49] W.T. Davis, T. Montrief, A. Koyfman, B. Long, Dysrhythmias and heart failure complicating acute myocardial infarction: an emergency medicine review, Am. J. Emerg. Med. 37 (2019) 1554–1561.
[50] H.L. Dauerman, B. Ibanez, The edge of time in acute myocardial infarction, J. Am. Coll. Cardiol. 77 (2021) 1871–1874.
[51] J. Frampton, A.R. Ortengren, E.P. Zeitler, Arrhythmias after acute myocardial infarction, Yale J. Biol. Med. 96 (2023) 83–94.
[52] P. Wu, M. Vaseghi, The autonomic nervous system and ventricular arrhythmias in myocardial infarction and heart failure, Pacing Clin. Electrophysiol.: PACE 43 (2020) 172–180.
[53] H.E. Driessen, T.A.B. van Veen, G.J.J. Boink, Emerging molecular therapies targeting myocardial infarction-related arrhythmias, EP Europace 19 (2016) 518–528.
[54] X. Peng, J. Zhou, X.-S. Wu, New strategies for myocardial infarction treatment, J. Cardiol. Therapy 4 (2017) 664–670.
[55] R. Marín-Juez, H. El-Sammak, C.S. Helker, A. Kamezaki, S.T. Mullapuli, S.-I. Bibli, M.J. Foglia, I. Fleming, K.D. Poss, D.Y. Stainier, Coronary revascularization during heart regeneration is regulated by epicardial and endocardial cues and forms a scaffold for cardiomyocyte repopulation, Dev. Cell 51 (2019) (503–515. e504).
[56] L. Zhang, T. Li, Y. Yu, K. Shi, Z. Bei, Y. Qian, Z. Qian, An injectable conductive hydrogel restores electrical transmission at myocardial infarct site to preserve cardiac function and enhance repair, Bioact. Mater. 20 (2023) 339–354.
[57] G. Swaroop, Post-myocardial infarction heart failure: a review on management of drug therapies, Cureus 14 (2022).
[58] J.P. Leach, J.F. Martin, Cardiomyocyte proliferation for therapeutic regeneration, Curr. Cardiol. Rep. 20 (2018) 1–8.
[59] M.R. Mehra, C.E. Canter, M.M. Hannan, M.J. Semigran, P.A. Uber, D.A. Baran, L. Danziger-Isakov, J.K. Kirklin, R. Kirk, S.S. Kushwaha, The 2016 International Society for Heart Lung Transplantation listing criteria for heart transplantation: a 10-year update, J. Heart Lung Transplant. 35 (2016) 1–23.
[60] A. Lala, J.C. Rowland, B.S. Ferket, A.C. Gelijns, E. Bagiella, S.P. Pinney, A. J. Moskowitz, M.A. Miller, F.D. Pagani, D.M. Mancini, Strategies of wait-listing for heart transplant vs durable mechanical circulatory support alone for patients with advanced heart failure, JAMA Cardiol. 5 (2020) 652–659.
[61] C. Balbi, K. Lodder, A. Costa, S. Moimas, F. Moccia, T. van Herwaarden, V. Rosti, F. Campagnoli, A. Palmeri, P. De Biasio, Reactivating endogenous mechanisms of cardiac regeneration via paracrine boosting using the human amniotic fluid stem cell secretome, Int. J. Cardiol. 287 (2019) 87–95.
[62] R. Rikhtegar, M. Pezeshkian, S. Dolati, N. Safaie, A.A. Rad, M. Mahdipour, M. Nouri, A.R. Jodati, M. Yousefi, Stem cells as therapy for heart disease: iPSCs, ESCs, CSCs, and skeletal myoblasts, Biomed. Pharmacother. 109 (2019) 304–313.
[63] K. Zhu, M. Wu, H. Lai, C. Guo, J. Li, Y. Wang, Y. Chen, C. Wang, J. Shi, Nanoparticle-enhanced generation of gene-transfected mesenchymal stem cells for in vivo cardiac repair, Biomaterials 74 (2016) 188–199.
[64] X. Zhang, H. Zhang, Y. Zhang, H. Huangfu, Y. Yang, Q. Qin, Y. Zhang, Y. Zhou, 3D printed reduced graphene oxide-GelMA hybrid hydrogel scaffolds for potential neuralized bone regeneration, J. Mater. Chem. B 11 (2023) 1288–1301.
[65] J. Meng, B. Xiao, F. Wu, L. Sun, B. Li, W. Guo, X. Hu, X. Xu, T. Wen, J. Liu, Co-axial fibrous scaffolds integrating with carbon fiber promote cardiac tissue regeneration post myocardial infarction, Mater. Today Bio. 16 (2022), 100415.
[66] G. Basara, S.G. Ozcebe, B.W. Ellis, P. Zorlutuna, Tunable human myocardium derived decellularized extracellular matrix for 3D bioprinting and cardiac tissue engineering, Gels 7 (2021) 70.
[67] P. Koti, N. Muselimyan, E. Mirdamadi, H. Asfour, N.A. Sarvazyan, Use of GelMA for 3D printing of cardiac myocytes and fibroblasts, J. 3D Print. Med. 3 (2019) 11–22.
[68] J. Liu, J. He, J. Liu, X. Ma, Q. Chen, N. Lawrence, W. Zhu, Y. Xu, S. Chen, Rapid 3D bioprinting of in vitro cardiac tissue models using human embryonic stem cell-derived cardiomyocytes, Bioprinting 13 (2019), e00040.
[69] N. Muzzio, S. Moya, G. Romero, Multifunctional scaffolds and synergistic strategies in tissue engineering and regenerative medicine, Pharmaceutics 13 (2021).
[70] H. Qu, H. Fu, Z. Han, Y. Sun, Biomaterials for bone tissue engineering scaffolds: a review, RSC Adv. 9 (2019) 26252–26262.
[71] V. Sharma, S.K. Dash, K. Govarthanan, R. Gahtori, N. Negi, M. Barani, R. Tomar, S. Chakraborty, S. Mathapati, D.K. Bishi, Recent advances in cardiac tissue engineering for the management of myocardium infarction, Cells 10 (2021) 2538.
[72] K. Veerubhotla, Y. Lee, C.H. Lee, Parametric optimization of 3D printed hydrogel-based cardiovascular stent, Pharm. Res. 38 (2021) 885–900.
[73] Y. Wang, G. Li, L. Yang, R. Luo, G. Guo, Development of innovative biomaterials and devices for the treatment of cardiovascular diseases, Adv. Mater. 34 (2022) 2201971.
[74] K.M. Mabry, S.Z. Payne, K.S. Anseth, Microarray analyses to quantify advantages of 2D and 3D hydrogel culture systems in maintaining the native valvular interstitial cell phenotype, Biomaterials 74 (2016) 31–41.
[75] Q. Sun, Z. Zhang, Z. Sun, The potential and challenges of using stem cells for cardiovascular repair and regeneration, Genes & Diseas 1 (2014) 113–119.
[76] Z. Zhu, D. Huangfu, Human pluripotent stem cells: an emerging model in developmental biology, Development 140 (2013) 705–717.
[77] T. Häneke, M. Sahara, Progress in bioengineering strategies for heart regenerative medicine, Int. J. Mol. Sci. 23 (2022) 3482.
[78] Q. Li, J. Wang, Q. Wu, N. Cao, H.-T. Yang, Perspective on human pluripotent stem cell-derived cardiomyocytes in heart disease modeling and repair, Stem Cells Transl. Med. 9 (2020) 1121–1128.
[79] S. Huang, Y. Yang, Q. Yang, Q. Zhao, X. Ye, Engineered circulatory scaffolds for building cardiac tissue, J. Thorac. Dis. 10 (2018) S2312–s2328.
[80] P. Kerscher, J.A. Kaczmarek, S.E. Head, M.E. Ellis, W.J. Seeto, J. Kim, S. Bhattacharya, V. Suppiramaniam, E.A. Lipke, Direct production of human cardiac tissues by pluripotent stem cell encapsulation in gelatin Methacryloyl, ACS Biomater. Sci. Eng. 3 (2017) 1499–1509.
[81] Z. Zhao, C. Vizetto-Duarte, Z.K. Moay, M.I. Setyawati, M. Rakshit, M. H. Kathawala, K.W. Ng, Composite hydrogels in three-dimensional in vitro models, Front. Bioeng. Biotechnol. 8 (2020) 611.
[82] C.-M. Moysidou, C. Barberio, R.M. Owens, Advances in engineering human tissue models, Front. Bioeng. Biotechnol. 8 (2021), 620962.
[83] N. Jalilinejad, M. Rabiee, N. Baheiraei, R. Ghahremanzadeh, R. Salarian, N. Rabiee, O. Akhavan, P. Zarrintaj, A. Hejna, M.R. Saeb, Electrically conductive carbon-based (bio)-nanomaterials for cardiac tissue engineering, Bioeng. Transl. Med. 8 (2023), e10347.
[84] A. Scafa Udriște, A.-G. Niculescu, L. Iliuță, T. Bajeu, A. Georgescu, A. M. Grumezescu, E. Bădilă, Progress in biomaterials for cardiac tissue engineering and regeneration, Polymers 15 (2023) 1177.
[85] P. Wang, Y. Sun, X. Shi, H. Shen, H. Ning, H. Liu, 3D printing of tissue engineering scaffolds: a focus on vascular regeneration, Bio-design Manufact. 4 (2021) 344–378.
[86] A.H. Nguyen, P. Marsh, L. Schmiess-Heine, P.J. Burke, A. Lee, J. Lee, H. Cao, Cardiac tissue engineering: state-of-the-art methods and outlook, J. Biol. Eng. 57 (2019) 1–21.
[87] M. Ghovvati, M. Kharaziha, R. Ardehali, N. Annabi, Recent advances in designing Electroconductive biomaterials for cardiac tissue engineering, Adv. Healthc. Mater. 11 (2022), 2200055.
[88] H. Cao, L. Duan, Y. Zhang, J. Cao, K. Zhang, Current hydrogel advances in physicochemical and biological response-driven biomedical application diversity, Signal Transduct. Target. Ther. 6 (2021) 426.
[89] Q. Chai, Y. Jiao, X. Yu, Hydrogels for biomedical applications: their characteristics and the mechanisms behind them, Gels 3 (2017) 6.
[90] X. Wei, C. Liu, X. Zhang, Z. Li, X. Wang, Y. Xu, J. Shi, Q. Sun, M.N. Routledge, D. Zhang, An integrated 3D cell-based electrochemical biosensor for neurotoxicity assessment of organophosphorus pesticides, Sensors Actuators B Chem. 376 (2023), 132941.
[91] A. Zielińska, P. Eder, L. Rannier, J.C. Cardoso, P. Severino, A.M. Silva, E.B. Souto, Hydrogels for Modified-release Drug Delivery Systems, Curr. Pharm. Des. 28 (2022) 609–618.
[92] J. Wang, W. Xu, W. Zhang, J. Da, L. Liu, X. Huang, C. Yang, Y. Zhan, H. Jin, Y. Li, UV cross-linked injectable non-swelling dihydrocaffeic acid grafted chitosan hydrogel for promoting wound healing, Carbohydr. Polym. 314 (2023), 120926.
[93] M.A. Sakr, K. Sakthivel, T. Hossain, S.R. Shin, S. Siddiqua, J. Kim, K. Kim, Recent trends in gelatin methacryloyl nanocomposite hydrogels for tissue engineering, J. Biomed. Mater. Res. A 110 (2022) 708–724.
[94] A. Zielińska, J. Karczewski, P. Eder, T. Kolanowski, M. Szalata, K. Wielgus, M. Szalata, D. Kim, S.R. Shin, R. Słomski, E.B. Souto, Scaffolds for drug delivery and tissue engineering: the role of genetics, J. Control. Relea.: Off. J. Control. Relea. Soc. 359 (2023) 207–223.
[95] D.-M. Radulescu, I.A. Neacsu, A.-M. Grumezescu, E. Andronescu, New insights of scaffolds based on hydrogels in tissue engineering, Polymers 14 (2022) 799.
[96] D. Zhu, Z. Li, K. Huang, T.G. Caranasos, J.S. Rossi, K. Cheng, Minimally invasive delivery of therapeutic agents by hydrogel injection into the pericardial cavity for cardiac repair, Nat. Commun. 12 (2021) 1412.
[97] M. Raab, D.E. Discher, Matrix rigidity regulates microtubule network polarization in migration, Cytoskeleton 74 (2017) 114–124.
[98] W.Y. Wang, A.T. Pearson, M.L. Kutys, C.K. Choi, M.A. Wozniak, B.M. Baker, C. S. Chen, Extracellular matrix alignment dictates the organization of focal adhesions and directs uniaxial cell migration, APL Bioeng. 2 (2018), 046107.
[99] S. Peressotti, G.E. Koehl, J.A. Goding, R.A. Green, Self-assembling hydrogel structures for neural tissue repair, ACS Biomater. Sci. Eng. 7 (2021) 4136–4163.
[100] K. Lv, Q. Li, L. Zhang, Y. Wang, Z. Zhong, J. Zhao, X. Lin, J. Wang, K. Zhu, C. Xiao, Incorporation of small extracellular vesicles in sodium alginate hydrogel as a novel therapeutic strategy for myocardial infarction, Theranostics 9 (2019) 7403.
[101] R. Parhi, Cross-linked hydrogel for pharmaceutical applications: a review, Adv. Pharmaceut. Bull. 7 (2017) 515–530.
[102] J.M. Unagolla, A.C. Jayasuriya, Hydrogel-based 3D bioprinting: a comprehensive review on cell-laden hydrogels, bioink formulations, and future perspectives, Appl. Mater. Today 18 (2020), 100479.
[103] X. Huang, Z. Huang, W. Gao, W. Gao, R. He, Y. Li, R. Crawford, Y. Zhou, L. Xiao, Y. Xiao, Current advances in 3D dynamic cell culture systems, Gels 8 (2022) 829.
[104] C.E. Cimenci, N.J. Blackburn, V. Sedlakova, J. Pupkaite, M. Munoz, B.H. Rotstein, D.A. Spiegel, E.I. Alarcon, E.J. Suuronen, Combined methylglyoxal scavenger and collagen hydrogel therapy prevents adverse remodeling and improves cardiac function post-myocardial infarction, Adv. Funct. Mater. 32 (2022) 2108630.
[105] P. Nezhad-Mokhtari, M. Akrami-Hasan-Kohal, M. Ghorbani, An injectable chitosan-based hydrogel scaffold containing gold nanoparticles for tissue engineering applications, Int. J. Biol. Macromol. 154 (2020) 198–205.
[106] F. Zhang, K. Qu, X. Li, C. Liu, L.S. Ortiz, K. Wu, X. Wang, N. Huang, Gelatin-based hydrogels combined with electrical stimulation to modulate neonatal rat cardiomyocyte beating and promote maturation, Bio-Design Manufact. 4 (2021) 100–110.

[107] H. Li, B. Yu, P. Yang, J. Zhan, X. Fan, P. Chen, X. Liao, C. Ou, Y. Cai, M. Chen, Injectable AuNP-HA matrix with localized stiffness enhances the formation of gap junction in engrafted human induced pluripotent stem cell-derived cardiomyocytes and promotes cardiac repair, Biomaterials 279 (2021), 121231.
[108] Y. Efraim, H. Sarig, N.C. Anavy, U. Sarig, E. de Berardinis, S.-Y. Chaw, M. Krishnamoorthi, J. Kalifa, H. Bogireddi, T.V. Duc, Biohybrid cardiac ECM-based hydrogels improve long term cardiac function post myocardial infarction, Acta Biomater. 50 (2017) 220–233.
[109] Y. Kambe, T. Yamaoka, Biodegradation of injectable silk fibroin hydrogel prevents negative left ventricular remodeling after myocardial infarction, Biomater. Sci. 7 (2019) 4153–4165.
[110] J. Song, Z. Chen, Z. Liu, Y. Yi, O. Tsigkou, J. Li, Y. Li, Controllable release of vascular endothelial growth factor (VEGF) by wheel spinning alginate/silk fibroin fibers for wound healing, Mater. Des. 212 (2021), 110231.
[111] G.N. Grover, N. Rao, K.L. Christman, Myocardial matrix–polyethylene glycol hybrid hydrogels for tissue engineering, Nanotechnology 25 (2013), 014011.
[112] Z. Fan, M. Fu, Z. Xu, B. Zhang, Z. Li, H. Li, X. Zhou, X. Liu, Y. Duan, P.-H. Lin, Sustained release of a peptide-based matrix metalloproteinase-2 inhibitor to attenuate adverse cardiac remodeling and improve cardiac function following myocardial infarction, Biomacromolecules 18 (2017) 2820–2829.
[113] S.A. Bernal-Chávez, S. Alcalá-Alcalá, Y. Tapia-Guerrero, J.J. Magaña, M.L. Del Prado-Audelo, G. Leyva-Gómez, Cross-linked polyvinyl alcohol-xanthan gum hydrogel fabricated by freeze/thaw technique for potential application in soft tissue engineering, RSC Adv. 12 (2022) 21713–21724.
[114] Y. Gao, K. Peng, S. Mitragotri, Covalently crosslinked hydrogels via step-growth reactions: crosslinking chemistries, polymers, and clinical impact, Adv. Mater. 33 (2021) 2006362.
[115] B. Peña, M. Laughter, S. Jett, T.J. Rowland, M.R. Taylor, L. Mestroni, D. Park, Injectable hydrogels for cardiac tissue engineering, Macromol. Biosci. 18 (2018) 1800079.
[116] A. Serafin, C. Murphy, M.C. Rubio, M.N. Collins, Printable alginate/gelatin hydrogel reinforced with carbon nanofibers as electrically conductive scaffolds for tissue engineering, Mater. Sci. Eng. C 122 (2021), 111927.
[117] J. Dienes, S. Browne, B. Farjun, J. Amaral Passipieri, E.L. Mintz, G. Killian, K. E. Healy, G.J. Christ, Semisynthetic hyaluronic acid-based hydrogel promotes recovery of the injured tibialis anterior skeletal muscle form and function, ACS Biomater. Sci. Eng. 7 (2021) 1587–1599.
[118] L.M. Ptaszek, R. Portillo Lara, E. Shirzaei Sani, C. Xiao, J. Roh, X. Yu, P. A. Ledesma, C. Hsiang Yu, N. Annabi, J.N. Ruskin, Gelatin methacryloyl bioadhesive improves survival and reduces scar burden in a mouse model of myocardial infarction, J. Am. Heart Assoc. 9 (2020), e014199.
[119] J.R. Choi, K.W. Yong, J.Y. Choi, A.C. Cowie, Recent advances in photo-crosslinkable hydrogels for biomedical applications, BioTechniques 66 (2019) 40–53.
[120] Y.S. Zhang, A. Arneri, S. Bersini, S.-R. Shin, K. Zhu, Z. Goli-Malekabadi, J. Aleman, C. Colosi, F. Busignani, V. Dell'Erba, Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip, Biomaterials 110 (2016) 45–59.
[121] K. Zhu, S.R. Shin, T. van Kempen, Y.C. Li, V. Ponraj, A. Nasajpour, S. Mandla, N. Hu, X. Liu, J. Leijten, Gold nanocomposite bioink for printing 3D cardiac constructs, Adv. Funct. Mater. 27 (2017) 1605352.
[122] S. Boularaoui, A. Shanti, M. Lanotte, S. Luo, S. Bawazir, S. Lee, N. Christoforou, K. A. Khan, C. Stefanini, Nanocomposite conductive bioinks based on low-concentration GelMA and MXene nanosheets/gold nanoparticles providing enhanced printability of functional skeletal muscle tissues, ACS Biomater. Sci. Eng. 7 (2021) 5810–5822.
[123] C.M.S. Neto, F.C. Lima, R.P. Morais, L.R.M. de Andrade, R. de Lima, M.V. Chaud, M.M. Pereira, R.L.C. de Albuquerque Júnior, J.C. Cardoso, A. Zielińska, E. B. Souto, S. Lima A, P. Severino, Rutin-functionalized multi-walled carbon nanotubes: molecular docking, physicochemistry and cytotoxicity in fibroblasts, Toxics 9 (2021).
[124] R.P. Morais, G.B. Novais, L.S. Sangenito, A.L.S. Santos, R. Priefer, M. Morsink, M. C. Mendonça, E.B. Souto, P. Severino, J.C. Cardoso, Naringenin-functionalized multi-walled carbon nanotubes: a potential approach for site-specific remote-controlled anticancer delivery for the treatment of lung Cancer cells, Int. J. Mol. Sci. 21 (2020) 4557.
[125] G.B. Novais, M.A. Dias, A.A.M. Santana, T.C. Batista, M.N. Marques, R.L. C. Albuquerque-Júnior, M.Z. Gomes, P. Severino, E.B. Souto, J.C. Cardoso, Isoflavones-functionalized single-walled and multi-walled carbon nanotubes: synthesis and characterization of new nanoarchitetonics for biomedical uses, J. Mol. Struct. 1294 (2023) 136351.
[126] Y. Xu, M. Patino Gaillez, R. Rothe, S. Hauser, D. Voigt, J. Pietzsch, Y. Zhang, Conductive hydrogels with dynamic reversible networks for biomedical applications, Adv. Healthc. Mater. 10 (2021) 2100012.
[127] M. Yadid, R. Feiner, T. Dvir, Gold nanoparticle-integrated scaffolds for tissue engineering and regenerative medicine, Nano Lett. 19 (2019) 2198–2206.
[128] P. Shukla, M. Mitruka, F. Pati, The effect of the synthetic route on the biophysiochemical properties of methacrylated gelatin (GelMA) based hydrogel for development of GelMA-based bioinks for 3D bioprinting applications, Materialia 25 (2022), 101542.
[129] A.I. Van Den Bulcke, B. Bogdanov, N. De Rooze, E.H. Schacht, M. Cornelissen, H. Berghmans, Structural and rheological properties of methacrylamide modified gelatin hydrogels, Biomacromolecules 1 (2000) 31–38.
[130] B.H. Lee, N. Lum, L.Y. Seow, P.Q. Lim, L.P. Tan, Synthesis and characterization of types A and B gelatin methacryloyl for bioink applications, Materials 9 (2016) 797.
[131] I. Pepelanova, K. Kruppa, T. Scheper, A. Lavrentieva, Gelatin-methacryloyl (GelMA) hydrogels with defined degree of functionalization as a versatile toolkit for 3D cell culture and extrusion bioprinting, Bioengineering 5 (2018) 55.
[132] J.W. Nichol, S.T. Koshy, H. Bae, C.M. Hwang, S. Yamanlar, A. Khademhosseini, Cell-laden microengineered gelatin methacrylate hydrogels, Biomaterials 31 (2010) 5536–5544.
[133] C. Colosi, S.R. Shin, V. Manoharan, S. Massa, M. Costantini, A. Barbetta, M. R. Dokmeci, M. Dentini, A. Khademhosseini, Microfluidic Bioprinting of Heterogeneous 3D Tissue Constructs Using Low-Viscosity Bioink, Advanced Materials (Deerfield Beach, Fla.) vol. 28, 2016, pp. 677–684.
[134] W. Liu, M.A. Heinrich, Y. Zhou, A. Akpek, N. Hu, X. Liu, X. Guan, Z. Zhong, X. Jin, A. Khademhosseini, Y.S. Zhang, Extrusion bioprinting of shear-thinning gelatin Methacryloyl bioinks, Adv. Healthc. Mater. 6 (2017).
[135] S. Bupphathong, C. Quiroz, W. Huang, P.F. Chung, H.Y. Tao, C.H. Lin, Gelatin Methacrylate Hydrogel for Tissue Engineering Applications-A Review on Material Modifications, Pharmaceuticals (Basel, Switzerland) vol. 15, 2022.
[136] W. Tomal, J. Ortyl, Water-soluble Photoinitiators in biomedical applications, Polymers 12 (2020) 1073.
[137] A.K. Nguyen, P.L. Goering, V. Reipa, R.J. Narayan, Toxicity and photosensitizing assessment of gelatin methacryloyl-based hydrogels photoinitiated with lithium phenyl-2,4,6-trimethylbenzoylphosphinate in human primary renal proximal tubule epithelial cells, Biointerphases 14 (2019), 021007.
[138] Z. Wang, X. Jin, R. Dai, J.F. Holzman, K. Kim, An ultrafast hydrogel photocrosslinking method for direct laser bioprinting, RSC Adv. 6 (2016) 21099–21104.
[139] I. Noshadi, S. Hong, K.E. Sullivan, E. Shirzaei Sani, R. Portillo-Lara, A. Tamayol, S. R. Shin, A.E. Gao, W.L. Stoppel, L.D. Black III, A. Khademhosseini, N. Annabi, In vitro and in vivo analysis of visible light crosslinkable gelatin methacryloyl (GelMA) hydrogels, Biomater. Sci. 5 (2017) 2093–2105.
[140] S. Sharifi, H. Sharifi, A. Akbari, J. Chodosh, Systematic optimization of visible light-induced crosslinking conditions of gelatin methacryloyl (GelMA), Sci. Rep. 11 (2021) 23276.
[141] H. Saini, A. Navaei, A. Van Putten, M. Nikkhah, 3D cardiac microtissues encapsulated with the co-culture of cardiomyocytes and cardiac fibroblasts, Adv. Healthc. Mater. 4 (2015) 1961–1971.
[142] L.E. Bertassoni, J.C. Cardoso, V. Manoharan, A.L. Cristino, N.S. Bhise, W. A. Araujo, P. Zorlutuna, N.E. Vrana, A.M. Ghaemmaghami, M.R. Dokmeci, A. Khademhosseini, Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels, Biofabrication 6 (2014), 024105.
[143] Y.-C. Chen, R.-Z. Lin, H. Qi, Y. Yang, H. Bae, J.M. Melero-Martin, A. Khademhosseini, Functional human vascular network generated in photocrosslinkable gelatin methacrylate hydrogels, Adv. Funct. Mater. 22 (2012) 2027–2039.
[144] J.A. Benton, C.A. DeForest, V. Vivekanandan, K.S. Anseth, Photocrosslinking of gelatin macromers to synthesize porous hydrogels that promote valvular interstitial cell function, Tissue Eng. Part A 15 (2009) 3221–3230.
[145] A.H. Sadeghi, S.R. Shin, J.C. Deddens, G. Fratta, S. Mandla, I.K. Yazdi, G. Prakash, S. Antona, D. Demarchi, M.P. Buijsrogge, Engineered 3D cardiac fibrotic tissue to study fibrotic remodeling, Adv. Healthc. Mater. 6 (2017) 1601434.
[146] P. Ravishankar, A. Ozkizilcik, A. Husain, K. Balachandran, Anisotropic Fiber-reinforced glycosaminoglycan hydrogels for heart valve tissue engineering, Tissue Eng. Part A 27 (2020) 513–525.
[147] J. Liu, K. Miller, X. Ma, S. Dewan, N. Lawrence, G. Whang, P. Chung, A. D. McCulloch, S. Chen, Direct 3D bioprinting of cardiac micro-tissues mimicking native myocardium, Biomaterials 256 (2020), 120204.
[148] S. Zhu, C. Yu, N. Liu, M. Zhao, Z. Chen, J. Liu, G. Li, H. Huang, H. Guo, T. Sun, J. Chen, J. Zhuang, P. Zhu, Injectable conductive gelatin methacrylate / oxidized dextran hydrogel encapsulating umbilical cord mesenchymal stem cells for myocardial infarction treatment, Bioact. Mater. 13 (2022) 119–134.
[149] G. Zu, M. Meijer, O. Mergel, H. Zhang, P. van Rijn, 3D-printable hierarchical nanogel-GelMA composite hydrogel system, Polymers 13 (2021) 2508.
[150] Z. Wang, H. Kumar, Z. Tian, X. Jin, J.F. Holzman, F. Menard, K. Kim, Visible light Photoinitiation of cell-adhesive gelatin Methacryloyl hydrogels for Stereolithography 3D bioprinting, ACS Appl. Mater. Interfaces 10 (2018) 26859–26869.
[151] P. Occhetta, R. Visone, L. Russo, L. Cipolla, M. Moretti, M. Rasponi, VA-086 methacrylate gelatine photopolymerizable hydrogels: a parametric study for highly biocompatible 3D cell embedding, J. Biomed. Mater. Res. A 103 (2015) 2109–2117.
[152] L.H. Kang, P.A. Armstrong, L.J. Lee, B. Duan, K.H. Kang, J.T. Butcher, Optimizing photo-encapsulation viability of heart valve cell types in 3D printable composite hydrogels, Ann. Biomed. Eng. 45 (2017) 360–377.
[153] S. Xiao, T. Zhao, J. Wang, C. Wang, J. Du, L. Ying, J. Lin, C. Zhang, W. Hu, L. Wang, Gelatin methacrylate (GelMA)-based hydrogels for cell transplantation: an effective strategy for tissue engineering, Stem Cell Rev. Rep. 15 (2019) 664–679.
[154] T. Liu, W. Weng, Y. Zhang, X. Sun, H. Yang, Applications of gelatin methacryloyl (GelMA) hydrogels in microfluidic technique-assisted tissue engineering, Molecules 25 (2020) 5305.
[155] K. Hölzl, M. Fürsatz, H. Göcerler, B. Schädl, S. Žigon-Branc, M. Markovic, C. Gahleitner, J.V. Hoorick, S. Van Vlierberghe, A. Kleiner, S. Baudis, A. Pauschitz, H. Redl, A. Ovsianikov, S. Nürnberger, Gelatin methacryloyl as environment for chondrocytes and cell delivery to superficial cartilage defects, J. Tissue Eng. Regen. Med. 16 (2022) 207–222.
[156] L. Fan, C. Liu, X. Chen, Y. Zou, Z. Zhou, C. Lin, G. Tan, L. Zhou, C. Ning, Q. Wang, Directing induced pluripotent stem cell derived neural stem cell fate with a three-

dimensional biomimetic hydrogel for spinal cord injury repair, ACS Appl. Mater. Interfaces 10 (2018) 17742–17755.
[157] C. Kilic Bektas, V. Hasirci, Cell loaded 3D bioprinted GelMA hydrogels for corneal stroma engineering, biomaterials, Science 8 (2020) 438–449.
[158] J. Zhang, W. Xu, C. Li, F. Meng, Y. Guan, X. Liu, J. Zhao, J. Peng, Y. Wang, Tissue engineering microtissue: construction, Optimiz. Appl. Tissue Eng. Part B: Rev. 28 (2022) 393–404.
[159] S. Mantha, S. Pillai, P. Khayambashi, A. Upadhyay, Y. Zhang, O. Tao, H.M. Pham, S.D. Tran, Smart hydrogels in tissue engineering and regenerative medicine, Materials 12 (2019) 3323.
[160] X.P. Li, K.Y. Qu, F. Zhang, H.N. Jiang, N. Zhang, N. Cheraga, C.M. Liu, K.H. Wu, X. W. Wang, N.P. Huang, Correction: high-aspect-ratio water-dispersed gold nanowires incorporated within gelatin methacrylate hydrogels for constructing cardiac tissues in vitro, J. Mater. Chem. B 8 (2020) 8085.
[161] R. Singla, S.M.S. Abidi, A.I. Dar, A. Acharya, Nanomaterials as potential and versatile platform for next generation tissue engineering applications, J. Biomed. Mater. Res. Part B 107 (2019) 2433–2449.
[162] S.R. Shin, S.M. Jung, M. Zalabany, K. Kim, P. Zorlutuna, S.B. Kim, M. Nikkhah, M. Khabiry, M. Azize, J. Kong, Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators, ACS Nano 7 (2013) 2369–2380.
[163] A. Paul, A. Hasan, H.A. Kindi, A.K. Gaharwar, V.T. Rao, M. Nikkhah, S.R. Shin, D. Krafft, M.R. Dokmeci, D. Shum-Tim, A. Khademhosseini, Injectable graphene oxide/hydrogel-based angiogenic gene delivery system for vasculogenesis and cardiac repair, ACS Nano 8 (2014) 8050–8062.
[164] S.R. Shin, C. Shin, A. Memic, S. Shadmehr, M. Miscuglio, H.Y. Jung, S.M. Jung, H. Bae, A. Khademhosseini, X. Tang, Aligned carbon nanotube–based flexible gel substrates for engineering biohybrid tissue actuators, Adv. Funct. Mater. 25 (2015) 4486–4495.
[165] A. Navaei, H. Saini, W. Christenson, R.T. Sullivan, R. Ros, M. Nikkhah, Gold nanorod-incorporated gelatin-based conductive hydrogels for engineering cardiac tissue constructs, Acta Biomater. 41 (2016) 133–146.
[166] S.R. Shin, C. Zihlmann, M. Akbari, P. Assawes, L. Cheung, K. Zhang, V. Manoharan, Y.S. Zhang, M. Yüksekkaya, K.T. Wan, Reduced graphene oxide-gelMA hybrid hydrogels as scaffolds for cardiac tissue engineering, Small 12 (2016) 3677–3689.
[167] S. Ahadian, S. Yamada, J. Ramón-Azcón, M. Estili, X. Liang, K. Nakajima, H. Shiku, A. Khademhosseini, T. Matsue, Hybrid hydrogel-aligned carbon nanotube scaffolds to enhance cardiac differentiation of embryoid bodies, Acta Biomater. 31 (2016) 134–143.
[168] A. Navaei, N. Moore, R.T. Sullivan, D. Truong, R.Q. Migrino, M. Nikkhah, Electrically conductive hydrogel-based micro-topographies for the development of organized cardiac tissues, RSC Adv. 7 (2017) 3302–3312.
[169] H. Sun, J. Tang, Y. Mou, J. Zhou, L. Qu, K. Duval, Z. Huang, N. Lin, R. Dai, C. Liang, Z. Chen, L. Tang, F. Tian, Carbon nanotube-composite hydrogels promote intercalated disc assembly in engineered cardiac tissues through β1-integrin mediated FAK and RhoA pathway, Acta Biomater. 48 (2017) 88–99.
[170] A. Navaei, K. Rahmani Eliato, R. Ros, R.Q. Migrino, B.C. Willis, M. Nikkhah, The influence of electrically conductive and non-conductive nanocomposite scaffolds on the maturation and excitability of engineered cardiac tissues, Biomater. Sci. 7 (2019) 585–595.
[171] B. Maharjan, D. Kumar, G.P. Awasthi, D.P. Bhattarai, J.Y. Kim, C.H. Park, C. S. Kim, Synthesis and characterization of gold/silica hybrid nanoparticles incorporated gelatin methacrylate conductive hydrogels for H9C2 cardiac cell compatibility study, Compos. Part B Eng. 177 (2019), 107415.
[172] J. Lee, V. Manoharan, L. Cheung, S. Lee, B.-H. Cha, P. Newman, R. Farzad, S. Mehrotra, K. Zhang, F. Khan, Nanoparticle-based hybrid scaffolds for deciphering the role of multimodal cues in cardiac tissue engineering, ACS Nano 13 (2019) 12525–12539.
[173] X.-P. Li, K.-Y. Qu, B. Zhou, F. Zhang, Y.-Y. Wang, O.D. Abodunrin, Z. Zhu, N.-P. Huang, Electrical stimulation of neonatal rat cardiomyocytes using conductive polydopamine-reduced graphene oxide-hybrid hydrogels for constructing cardiac microtissues, Colloids Surf. B: Biointerfaces 205 (2021), 111844.
[174] L. Sun, X. Zhu, X. Zhang, G. Chen, F. Bian, J. Wang, Q. Zhou, D. Wang, Y. Zhao, Induced cardiomyocytes-integrated conductive microneedle patch for treating myocardial infarction, Chem. Eng. J. 414 (2021), 128723.
[175] H.-L. Tan, S.-Y. Teow, J. Pushpamalar, Application of metal nanoparticle–hydrogel composites in tissue regeneration, Bioengineering 6 (2019) 17.
[176] K. Hosoyama, M. Ahumada, C.D. McTiernan, J. Bejjani, F. Variola, M. Ruel, B. Xu, W. Liang, E.J. Suuronen, E.I. Alarcon, Multi-functional thermo-crosslinkable collagen-metal nanoparticle composites for tissue regeneration: nanosilver vs. nanogold, RSC Adv. 7 (2017) 47704–47708.
[177] L. Zwi-Dantsis, B. Wang, C. Marijon, S. Zonetti, A. Ferrini, L. Massi, D.J. Stuckey, C.M. Terracciano, M.M. Stevens, Remote magnetic nanoparticle manipulation enables the dynamic patterning of cardiac tissues, Adv. Mater. (Deerfield Beach, Fla.) 32 (2020) e1904598.
[178] K. Roshanbinfar, Z. Mohammadi, A. Sheikh-Mahdi Mesgar, M.M. Dehghan, O. P. Oommen, J. Hilborn, F.B. Engel, Carbon nanotube doped pericardial matrix derived electroconductive biohybrid hydrogel for cardiac tissue engineering, biomaterials, Science 7 (2019) 3906–3917.
[179] M.H. Norahan, M. Pourmokhtari, M.R. Saeb, B. Bakhshi, M. Soufi Zomorrod, N. Baheiraei, Electroactive cardiac patch containing reduced graphene oxide with potential antibacterial properties, Mater. Sci. Eng. C 104 (2019), 109921.
[180] B. Li, S. Dong, X. Wu, C. Wang, X. Wang, J. Fang, Anisotropic thermal property of magnetically oriented carbon nanotube/graphene polymer composites, Compos. Sci. Technol. 147 (2017) 52–61.
[181] S.R. Shin, H. Bae, J.M. Cha, J.Y. Mun, Y.-C. Chen, H. Tekin, H. Shin, S. Zarabi, M. R. Dokmeci, S. Tang, Carbon nanotube reinforced hybrid microgels as scaffold materials for cell encapsulation, ACS Nano 6 (2011) 362–372.
[182] S. Pacelli, K. Rampetsreiter, S. Modaresi, S. Subham, A.R. Chakravarti, S. Lohfeld, M.S. Detamore, A. Paul, Fabrication of a double-cross-linked interpenetrating polymeric network (IPN) hydrogel surface modified with polydopamine to modulate the osteogenic differentiation of adipose-derived stem cells, ACS Appl. Mater. Interfaces 10 (2018) 24955–24962.
[183] M. Shevach, S. Fleischer, A. Shapira, T. Dvir, Gold nanoparticle-decellularized matrix hybrids for cardiac tissue engineering, Nano Lett. 14 (2014) 5792–5796.
[184] M. Lee, M.C. Kim, J.Y. Lee, Nanomaterial-based electrically conductive hydrogels for cardiac tissue repair, Int. J. Nanomedicine 17 (2022) 6181–6200.
[185] S.R. Shin, B. Aghaei-Ghareh-Bolagh, T.T. Dang, S.N. Topkaya, X. Gao, S.Y. Yang, S.M. Jung, J.H. Oh, M.R. Dokmeci, X. Tang, Cell-laden microengineered and mechanically tunable hybrid hydrogels of gelatin and graphene oxide, Adv. Mater. 25 (2013) 6385–6391.
[186] M. Morsink, P. Severino, E. Luna-Ceron, M.A. Hussain, N. Sobahi, S.R. Shin, Effects of electrically conductive nano-biomaterials on regulating cardiomyocyte behavior for cardiac repair and regeneration, Acta Biomater. 139 (2022) 141–156.
[187] R.A. D, E. Sink, S.P. Narayan, M. Abdel-Hafiz, L. Mestroni, B. Peña, Nanomaterials for cardiac tissue engineering, Molecules 25 (2020).
[188] G. Zhao, Y. Feng, L. Xue, M. Cui, Q. Zhang, F. Xu, N. Peng, Z. Jiang, D. Gao, X. Zhang, Anisotropic conductive reduced graphene oxide/silk matrices promote post-infarction myocardial function by restoring electrical integrity, Acta Biomater. 139 (2022) 190–203.
[189] A. Saberi, F. Jabbari, P. Zarrintaj, M.R. Saeb, M. Mozafari, Electrically conductive materials: opportunities and challenges in tissue engineering, Biomolecules 9 (2019) 448.
[190] H.-T. Shi, Z.-H. Huang, T.-Z. Xu, A.-J. Sun, J.-B. Ge, New diagnostic and therapeutic strategies for myocardial infarction via nanomaterials, EBioMedicine 78 (2022), 103968.
[191] Q.A. Majid, A.T. Fricker, D.A. Gregory, N. Davidenko, O. Hernandez Cruz, R. J. Jabbour, T.J. Owen, P. Basnett, B. Lukasiewicz, M. Stevens, Natural biomaterials for cardiac tissue engineering: a highly biocompatible solution, Front. Cardiovasc. Med. 192 (2020).
[192] H. Esmaeili, A. Patino-Guerrero, M. Hasany, M.O. Ansari, A. Memic, A. Dolatshahi-Pirouz, M. Nikkhah, Electroconductive biomaterials for cardiac tissue engineering, Acta Biomater. 139 (2022) 118–140.
[193] X. Hu, Y. Zhang, T. Ding, J. Liu, H. Zhao, Multifunctional gold nanoparticles: a novel nanomaterial for various medical applications and biological activities, Front. Bioeng. Biotechnol. 8 (2020) 990.
[194] I. Hammami, N.M. Alabdallah, A.A. Jomaa, M. Kamoun, Gold nanoparticles: synthesis properties and applications, J. King Saud Univer. - Sci. 33 (2021), 101560.
[195] Z. Niknam, F. Hosseinzadeh, F. Shams, L. Fath-Bayati, G. Nuoroozi, L. Mohammadi Amirabad, F. Mohebichamkhorami, S. Khakpour Naeimi, S. Ghafouri-Fard, H. Zali, Recent advances and challenges in graphene-based nanocomposite scaffolds for tissue engineering application, J. Biomed. Mater. Res. A 110 (2022) 1695–1721.
[196] E. Tahmasebi, M. Alam, M. Yazdanian, H. Tebyanian, A. Yazdanian, A. Seifalian, S.A. Mosaddad, Current biocompatible materials in oral regeneration: a comprehensive overview of composite materials, J. Mater. Res. Technol. 9 (2020) 11731–11755.
[197] Y. Zhang, C. Wu, S. Guo, J. Zhang, Interactions of graphene and graphene oxide with proteins and peptides, Nanotechnol. Rev. 2 (2013) 27–45.
[198] A. Jiříčková, O. Jankovský, Z. Sofer, D. Sedmidubský, Synthesis and applications of graphene oxide, Materials, 2022.
[199] A. Raslan, L. Saenz Del Burgo, J. Ciriza, J.L. Pedraz, Graphene oxide and reduced graphene oxide-based scaffolds in regenerative medicine, Int. J. Pharm. 580 (2020), 119226.
[200] V. Agarwal, P.B. Zetterlund, Strategies for reduction of graphene oxide – a comprehensive review, Chem. Eng. J. 405 (2021), 127018.
[201] M. Solazzo, F.J. O'Brien, V. Nicolosi, M.G. Monaghan, The rationale and emergence of electroconductive biomaterial scaffolds in cardiac tissue engineering, APL Bioeng. 3 (2019), 041501.
[202] K. Yue, G. Trujillo-de Santiago, M.M. Alvarez, A. Tamayol, N. Annabi, A. Khademhosseini, Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels, Biomaterials 73 (2015) 254–271.
[203] E. Singh, R. Srivastava, U. Kumar, A.D. Katheria, Carbon nanotube: a review on introduction, Fabric. Techn. Optical Appl. Nanosci. Nanotechnol. Res. 4 (2017) 120–126.
[204] R.B. Onyancha, U.O. Aigbe, K.E. Ukhurebor, P.W. Muchiri, Facile synthesis and applications of carbon nanotubes in heavy-metal remediation and biomedical fields: a comprehensive review, J. Mol. Struct. 1238 (2021), 130462.
[205] N. Kittana, M. Assali, H. Abu-Rass, S. Lutz, R. Hindawi, L. Ghannam, M. Zakarneh, A. Mousa, Enhancement of wound healing by single-wall/multi-wall carbon nanotubes complexed with chitosan, Int. J. Nanomedicine 13 (2018) 7195–7206.
[206] A. Ul Haq, F. Carotenuto, F. Trovalusci, F. De Matteis, P. Di Nardo, Carbon nanomaterials-based electrically conductive scaffolds to repair the Ischaemic heart tissue, J. Carbon Res. 8 (2022).
[207] J. Liu, T. Zou, Y. Zhang, J. Koh, H. Li, Y. Wang, Y. Zhao, C. Zhang, Three-dimensional electroconductive carbon nanotube-based hydrogel scaffolds enhance neural differentiation of stem cells from apical papilla, Biomater. Adv. 138 (2022), 212868.

[208] H. Sun, J. Zhou, Z. Huang, L. Qu, N. Lin, C. Liang, R. Dai, L. Tang, F. Tian, Carbon nanotube-incorporated collagen hydrogels improve cell alignment and the performance of cardiac constructs, Int. J. Nanomedicine 12 (2017) 3109–3120.
[209] B. Yang, L. Gao, M. Xue, H. Wang, Y. Hou, Y. Luo, H. Xiao, H. Hu, C. Cui, H. Wang, J. Zhang, Y.F. Li, G. Xie, X. Tong, Y. Xie, Experimental and simulation research on the preparation of carbon nano-materials by chemical vapor deposition, Mater. (Basel, Switzerl.) 14 (2021).
[210] J. Ren, Q. Xu, X. Chen, W. Li, K. Guo, Y. Zhao, Q. Wang, Z. Zhang, H. Peng, Y. G. Li, Superaligned carbon nanotubes guide oriented cell growth and promote electrophysiological homogeneity for synthetic cardiac tissues, Adv. Mater. (Deerfield Beach, Fla.) 29 (2017).
[211] T. Jain, H.B. Baker, A. Gipsov, J.P. Fisher, A. Joy, D.S. Kaplan, I. Isayeva, Impact of cell density on the bioprinting of gelatin methacrylate (GelMA) bioinks, Bioprinting 22 (2021), e00131.
[212] M.B. Chen, S. Srigunapalan, A.R. Wheeler, C.A. Simmons, A 3D microfluidic platform incorporating methacrylated gelatin hydrogels to study physiological cardiovascular cell–cell interactions, Lab Chip 13 (2013) 2591–2598.
[213] L. Sun, Z. Chen, D. Xu, Y. Zhao, Electroconductive and anisotropic structural color hydrogels for visual heart-on-a-Chip construction, Adv. Sci. 9 (2022) 2105777.
[214] A.L.Y. Nachlas, S. Li, B.W. Streeter, K.J. De Jesus Morales, F. Sulejmani, D. I. Madukauwa-David, D. Bejleri, W. Sun, A.P. Yoganathan, M.E. Davis, A multilayered valve leaflet promotes cell-laden collagen type I production and aortic valve hemodynamics, Biomaterials 240 (2020), 119838.
[215] L. Shao, Q. Gao, C. Xie, J. Fu, M. Xiang, Y. He, Directly coaxial 3D bioprinting of large-scale vascularized tissue constructs, Biofabrication 12 (2020), 035014.
[216] M.T. Tavares, V.M. Gaspar, M.V. Monteiro, J.P.S. Farinha, C. Baleizão, J.F. Mano, GelMA/bioactive silica nanocomposite bioinks for stem cell osteogenic differentiation, Biofabrication 13 (2021), 035012.
[217] X. Hou, Y. Chen, F. Chen, J. Liu, T. Wang, Y. Luo, S. Jia, P. Wang, S. Tan, B. Lu, Z. Zhou, L. Zheng, The hydroxyapatite microtubes enhanced GelMA hydrogel scaffold with inner "pipeline framework" structure for bone tissue regeneration, Compos. Part B Eng. 228 (2022), 109396.
[218] I. Jahan, E. George, N. Saxena, S. Sen, Silver-nanoparticle-entrapped soft GelMA gels as prospective scaffolds for wound healing, ACS Appl. Bio Mater. 2 (2019) 1802–1814.
[219] Y. Wang, Y. Xu, L. Shang, Y. Mao, GelMA hydrogel scaffold containing curcumin-loaded solid lipid nanoparticles promotes the regeneration of degenerative discs, SN Appl. Sci. 5 (2023) 243.
[220] Y. Hu, Y. Jia, H. Wang, Q. Cao, Y. Yang, Y. Zhou, T. Tan, X. Huang, Q. Zhou, Low-intensity pulsed ultrasound promotes cell viability and inhibits apoptosis of H9C2 cardiomyocytes in 3D bioprinting scaffolds via PI3K-Akt and ERK1/2 pathways, J. Biomater. Appl. 37 (2022) 402–414.
[221] M. Kitsara, D. Kontziampasis, O. Agbulut, Y. Chen, Heart on a chip: Micro-nanofabrication and microfluidics steering the future of cardiac tissue engineering, Microelectron. Eng. 203-204 (2019) 44–62.
[222] Q. Yang, Z. Xiao, X. Lv, T. Zhang, H. Liu, Fabrication and biomedical applications of heart-on-a-chip, Intern. J. Bioprint. 7 (2021) 370.
[223] Y.S. Zhang, Q. Pi, A.M. van Genderen, Microfluidic bioprinting for engineering vascularized tissues and organoids, J. Vis. Exp.: JoVE 126 (2017) 55957.
[224] T. Su, K. Huang, M.A. Daniele, M.T. Hensley, A.T. Young, J. Tang, T.A. Allen, A. C. Vandergriff, P.D. Erb, F.S. Ligler, K. Cheng, Cardiac stem cell patch integrated with microengineered blood vessels promotes cardiomyocyte proliferation and neovascularization after acute myocardial infarction, ACS Appl. Mater. Interfaces 10 (2018) 33088–33096.
[225] E.P. Chen, Z. Toksoy, B.A. Davis, J.P. Geibel, 3D bioprinting of vascularized tissues for in vitro and in vivo applications, Front. Bioeng. Biotechnol. 9 (2021) (Article 664188).
[226] G. Vunjak-Novakovic, N. Tandon, A. Godier, R. Maidhof, A. Marsano, T. P. Martens, M. Radisic, Challenges in cardiac tissue engineering, Tiss. Eng. Part B. Rev. 16 (2010) 169–187.
[227] A. Akbarzadeh, S. Sobhani, A. Soltani Khaboushan, A.M. Kajbafzadeh, Whole-heart tissue engineering and cardiac patches: challenges and promises, Bioeng. (Basel, Switzerl.) vol. 10 (2023).