MEDICINE AND MATERIALS

Volume 6, Issue 1, 2026: 3-10 | ISSN: 2784 – 1499 & e-ISSN: 2784 – 1537
DOI: 10.36868/MEDMATER.2026.06.01.01 | www.medicineandmaterials.com |

ASSESSMENT OF CELLULAR VIABILITY AND BIOCOMPATIBILITY IN CARDIAC-RELEVANT CELL CULTURES

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Abstract

Cardiac tissue regeneration remains a major challenge due to the limited regenerative capacity of adult myocardial tissue. In this context, biomaterial-based strategies have gained increasing attention for their ability to modulate cellular behavior under controlled in vitro conditions. The present study aimed to comparatively evaluate four biomaterial categories relevant for cardiac regeneration: polymeric scaffolds, injectable hydrogels, carbon-based nanomaterials, and bioactive growth factor-functionalized systems. Cardiac-relevant cells were cultured in contact with the investigated biomaterials, and cellular viability, adhesion, morphology, and functional response were systematically assessed. Our in vitro results demonstrated material-dependent cellular responses across all evaluated parameters. Polymeric scaffolds and injectable hydrogels exhibited favorable biocompatibility profiles and supported sustained cell survival and adhesion. Carbon-based nanomaterials induced a dose-dependent cellular response, highlighting the importance of concentration optimization. Bioactive biomaterials showed enhanced cellular viability and functional activity, emphasizing the role of biochemical cues in cardiac tissue engineering. Overall, our findings underline the critical influence of biomaterial composition and functionalization on cellular behavior and provide a comparative in vitro framework to support rational biomaterial selection for cardiac regeneration, oriented applications.

Keywords: cardiac tissue engineering, biomaterials, polymeric scaffolds, injectable hydrogels, carbon-based nanomaterials, bioactive materials.

Introduction

Cardiovascular diseases remain the leading cause of morbidity and mortality worldwide, creating a persistent need for innovative regenerative strategies capable of restoring damaged myocardial tissue. Conventional therapies are often limited to symptom control and do not adequately address the irreversible loss of functional cardiomyocytes following injury. In this context, cardiac tissue engineering has emerged as a promising approach, integrating biomaterials and cell-based strategies to support myocardial regeneration under controlled in vitro conditions [1].

Among the approaches investigated by us, polymer scaffolding has attracted significant attention due to its ability to mimic the architecture of the extracellular matrix and provide

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mechanical support for cardiac cells. Early in vitro studies have demonstrated that degradable scaffolding with biomimetic topography can support cardiomyocyte attachment and function, highlighting their potential for altered heart grafts [1,2]. Advances in scaffolding manufacturing, including electrocentrifugation and nanofibrous coaxial designs, have further improved cell alignment and structural integration, key factors for cardiac repair [2]. More recently, additive manufacturing techniques such as volumetric 3D printing and melt-electrowriting have enabled the development of reinforced cardiac tissue patches with increased structural fidelity [3,4].

Injectable hydrogel-based biomaterials represent an alternative strategy, offering minimally invasive delivery and the capacity to encapsulate cells within a three-dimensional microenvironment. Hydrogels with tunable mechanical properties have been shown to influence cardiomyocyte differentiation and maturation in vitro [4]. Fully defined alginate and hyaluronic acid hydrogels further improved reproducibility and cross-linking control for myocardial applications [5-7]. Functionalization of injectable systems with bioactive molecules, including stromal cell-derived factors and nanoformulations, has been reported to enhance cellular engraftment and regenerative potential, while maintaining acceptable biocompatibility profiles [6-8]. In parallel, carbon-based nanomaterials have been increasingly explored for cardiac tissue engineering due to their unique electrical and mechanical properties. Platforms incorporating carbon nanotubes or graphene derivatives demonstrated improved cardiomyocyte performance, electrical coupling, and functional maturation in vitro [9-12]. More recent developments integrating nanomaterials into injectable or decellularized matrices further expanded their applicability, although cellular responses remain strongly dependent on concentration and material formulation [13].

Bioactive biomaterials incorporating growth factors have also been investigated as a means to actively modulate cellular behavior. Collagen-based patches functionalized with vascular endothelial growth factor demonstrated enhanced cellular responses relevant to myocardial repair [14]. Engineered cardiac constructs and advanced hydrogel systems further emphasized the importance of biochemical cues for functional tissue development [15,16]. Additionally, emerging scaffold designs incorporating piezoelectric or conductive properties highlighted the relevance of mechanical and electrical stimulation in guiding cardiomyocyte organization and activity [17,18].

Our study aimed to compare, under in vitro standardized conditions, four representative categories of biomaterials relevant to cardiac regeneration: polymer scaffolding, injectable hydrogels, carbon-based nanomaterials, and functional systems with bioactive growth factors. By systematically evaluating cell viability, adhesion, morphology, and functional response, our work aims to provide an integrated experimental perspective on material-dependent cellular behavior in cardiac tissue engineering applications.

Materials and methods

Biomaterials preparation

We investigated four categories of biomaterials relevant to cardiac tissue regeneration: polymer scaffolding, hydrogel-based injectable biomaterials, carbon-based nanomaterials, and functional bioactive biomaterials with growth factors. All materials were prepared under sterile conditions before in vitro testing. Polymeric scaffolds were fabricated to obtain a porous three-dimensional structure, while injectable hydrogels were prepared to allow homogeneous cell encapsulation. Carbon-based nanomaterials were dispersed at predefined concentrations to assess dose-dependent cellular responses. Bioactive biomaterials were functionalized with growth factors incorporated during the preparation phase to ensure sustained bioavailability.

Cell culture conditions

Cardiac-relevant cells were cultured under standard in vitro conditions (37°C, 5% CO_2, humidified atmosphere) using a complete growth medium appropriate for cardiac tissue applications. Cells were expanded until reaching the desired confluence and subsequently seeded onto or within the investigated biomaterials at standardized densities. All experiments were conducted in triplicate to ensure reproducibility.

Cellular viability and biocompatibility assessment

Cell viability was assessed using metabolic activity-based assays following 24, 48, and 72 hours of incubation. Metabolic activity was quantified spectrophotometrically and expressed as a percentage relative to control cultures. These assays were used to evaluate cytocompatibility and to identify potential material-dependent differences in cellular tolerance. Viability values above 75% were considered indicative of acceptable in vitro biocompatibility.

Cell adhesion and morphological analysis

Cell adhesion was evaluated after 24-72 hours of culture by quantifying the proportion of adherent cells relative to the initial seeding density. Cellular morphology and spreading behavior were assessed using microscopic analysis. Qualitative parameters, including cell shape, alignment, and cytoskeletal organization, were recorded to characterize cell–material interactions across different biomaterial architectures. In addition, cell spreading profile and cytoskeletal organization were described using predefined semi-quantitative descriptive categories based on microscopic evaluation under standardized experimental conditions.

Functional cellular response and bioactivity evaluation

Functional cellular response was analyzed through proliferation assays and qualitative assessment of cardiac-relevant functional marker expression after 72 hours of exposure to the biomaterials. Proliferation rates were calculated relative to baseline values, while bioactivity levels were determined based on the overall capacity of each biomaterial to modulate cellular functional behavior. Functional marker expression and overall bioactivity were classified using descriptive semi-quantitative categories derived from the observed in vitro cellular response under identical culture conditions.

Statistical Analysis

Quantitative data obtained from the in vitro experiments are presented as mean ± standard deviation (SD). All experiments were performed in triplicate to ensure data reliability. Statistical analyses were conducted to evaluate differences between biomaterial groups using appropriate comparative tests. Differences were considered statistically significant at a threshold of $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics software, version 26.0, Build 1.0.0.1347 (IBM Corp., Armonk, NY, USA). Statistical analysis was performed using standard data analysis software. Qualitative and semi-quantitative morphological and functional descriptors were summarized descriptively and were not subjected to inferential statistical testing.

Results

The results of our in vitro study demonstrated distinct biomaterial-dependent effects on cell viability, adhesion, morphology, and functional response in the biomaterial categories investigated.

Cell viability and biocompatibility

The investigated biomaterials showed distinct in vitro viability profiles in cardiac-relevant cell cultures. Cell viability was assessed using metabolic activity-based assays after 24-72 h of exposure, and results are presented as mean ± standard deviation. According to the predefined threshold applied in this study, viability values above 75% were considered indicative of acceptable in vitro biocompatibility. Among the tested biomaterials, growth factor-functionalized bioactive systems exhibited the highest mean viability, followed by polymeric scaffolds and injectable hydrogels. Carbon-based nanomaterials showed the lowest viability values, although these remained within the range considered compatible with acceptable cellular tolerance. A dose-dependent effect was noted only in the carbon-based nanomaterial group (Table 1).

Table 1. Comparative ranking of biomaterial-associated cell viability and biocompatibility outcomes

Biomaterial category	Exposure interval	Cell viability (%), mean ± SD	Relative viability ranking	Biocompatibility profile	Dose-dependent response
Bioactive biomaterials (growth factor-functionalized)	24-72 h	96.1 ± 3.5	1	Highly favorable	No
Polymeric scaffolds	24-72 h	92.4 ± 4.1	2	Favorable	No
Injectable biomaterials (hydrogels)	24-72 h	89.7 ± 5.3	3	Favorable	No
Carbon-based nanomaterials	24-72 h	78.2 ± 6.8	4	Acceptable	Yes

Note: Ranking was established according to the mean cell viability values recorded after 24-72 h of exposure.

The comparative distribution of cell viability values across the investigated biomaterial categories is illustrated in Figure 1.

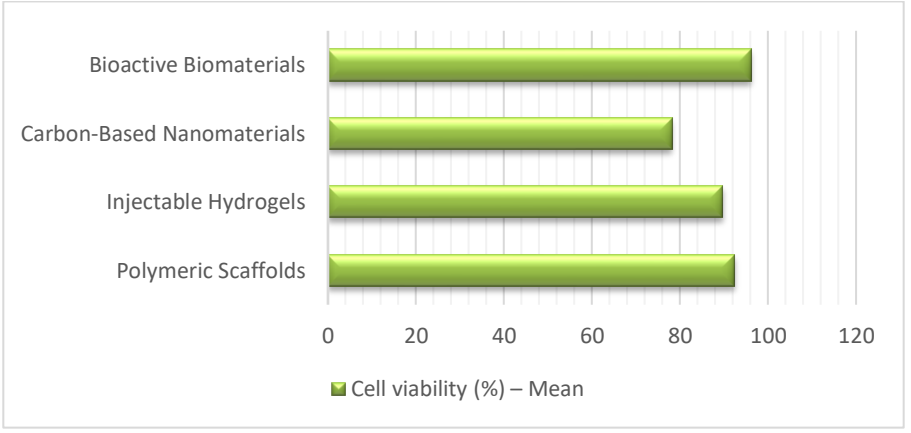

Figure 1. Comparative in vitro cellular viability of cardiac-relevant cells exposed to the investigated biomaterials. Data are expressed as mean ± SD.

Cell adhesion and morphological behavior

Cell adhesion analysis showed a pattern consistent with the viability findings. Bioactive biomaterials exhibited the highest adhesion rate (93.8 ± 3.9%), followed by polymeric scaffolds

(88.6 ± 5.0%) and injectable hydrogels (84.2 ± 6.1%), whereas carbon-based nanomaterials showed the lowest adhesion values (72.5 ± 7.4%). Microscopic examination supported these findings, showing very high spreading and highly organized cytoskeletal architecture in cells cultured on bioactive biomaterials. Polymeric scaffolds promoted high spreading and well-organized morphology, while injectable hydrogels showed moderate-to-high spreading with moderately organized cytoskeletal features. In contrast, carbon-based nanomaterials were associated with moderate spreading and more variable cytoskeletal organization (Table 2).

Table 2. Cell adhesion and morphological characteristics of cardiac-relevant cells cultured on the investigated biomaterials

Biomaterial category	Cell adhesion (%) mean ± SD	Relative adhesion ranking	Cell spreading	Cytoskeletal organization
Bioactive biomaterials (growth factor-functionalized)	93.8 ± 3.9	1	Very high	Highly organized
Polymeric scaffolds	88.6 ± 5.0	2	High	Well-organized
Injectable biomaterials (hydrogels)	84.2 ± 6.1	3	Moderate-to-high	Moderately organized
Carbon-based nanomaterials	72.5 ± 7.4	4	Moderate	Variable

Note: Relative adhesion ranking was established according to the mean cell adhesion values. Morphological characteristics were assessed by microscopic examination after 24-72 h of in vitro culture.

Functional cellular response

Functional cellular response was evaluated in vitro by proliferation assays and qualitative assessment of cardiac-relevant functional marker expression after 72 h of exposure to the investigated biomaterials. Results are presented as mean ± standard deviation. Among the tested biomaterial categories, growth factor-functionalized bioactive biomaterials showed the highest proliferation rate (97.2 ± 3.1%), followed by injectable hydrogels (85.9 ± 5.4%) and polymeric scaffolds (81.5 ± 6.2%). Carbon-based nanomaterials exhibited the lowest proliferation rate (69.8 ± 7.1%). A similar distribution was observed for functional marker expression and overall bioactivity profile. Bioactive biomaterials demonstrated high marker expression and very high bioactivity, whereas injectable hydrogels showed a moderate-to-high functional profile. Polymeric scaffolds were associated with moderate functional marker expression and moderate bioactivity, while carbon-based nanomaterials displayed the least favorable and most variable functional response among the investigated groups (Table 3). Taken together, these findings indicate that biomaterial composition and functionalization influenced not only cellular survival and adhesion, but also the capacity of the investigated systems to support early functional cellular activity in vitro. The most favorable profile was observed for bioactive biomaterials, while carbon-based nanomaterials showed the lowest overall functional performance.

Overall comparative pattern across biomaterial categories

When the evaluated parameters were considered together, a consistent comparative pattern emerged across the investigated biomaterial categories. Bioactive biomaterials showed the most favorable overall profile, with the highest values for cell viability, adhesion, and proliferation, together with the most favorable qualitative morphological and functional characteristics. Polymeric scaffolds and injectable hydrogels also demonstrated generally

favorable responses, whereas carbon-based nanomaterials consistently showed the lowest values and the greatest variability across the assessed parameters.

Table 3. Functional cellular response and bioactivity profile of cardiac-relevant cells exposed to the investigated biomaterials

Biomaterial category	Cell Proliferation rate (%) mean ± SD	Relative proliferation ranking	Functional marker expression	Bioactivity level
Bioactive biomaterials (growth factor-functionalized)	97.2 ± 3.1	1	High	Very high
Injectable biomaterials (hydrogels)	85.9 ± 5.4	2	Moderate-to-high	High
Polymeric scaffolds	81.5 ± 6.2	3	Moderate	Moderate
Carbon-based nanomaterials	69.8 ± 7.1	4	Low-to-moderate	Variable

Note: Relative proliferation ranking was established according to the mean proliferation values recorded after 72 h of exposure. Functional marker expression and bioactivity level were based on the qualitative assessment of cellular functional behavior under the experimental conditions used in this study

Discussion

Our study in vitro provides a comparative evaluation of four biomaterial categories widely investigated for cardiac tissue regeneration, focusing on their influence on cellular viability, adhesion, morphology, and functional response. By applying standardized experimental conditions, our results highlight distinct material-dependent cellular behaviors, offering an integrated perspective relevant for early-stage cardiac biomaterial screening.

In our study, polymeric scaffolds demonstrated favorable cytocompatibility, supporting high cellular viability and well-organized adhesion patterns. These findings are consistent with previous observations showing that scaffold architecture and extracellular matrix–like topography play a critical role in sustaining cardiomyocyte attachment and function [2]. Advanced fabrication strategies, such as electrospinning and additive manufacturing, have been shown to enhance structural guidance and mechanical stability, which may explain the stable cellular responses observed in our experiments [3-5]. The preserved cellular morphology and progressive proliferation noted in our scaffold-based systems further support their suitability as three-dimensional platforms for cardiac tissue engineering.

Injectable hydrogel-based biomaterials also exhibited high biocompatibility in our in vitro model, with sustained cell survival and homogeneous cell distribution. The capacity of hydrogels to provide a hydrated, three-dimensional microenvironment likely contributed to the moderate-to-high adhesion and proliferation rates observed in our study. Similar in vitro investigations have emphasized the importance of tunable mechanical properties and controlled cross-linking in guiding cardiomyocyte differentiation and maturation [4]. Moreover, hydrogel functionalization strategies incorporating bioactive molecules or nanoformulations have been reported to enhance regenerative outcomes, in line with the increased functional cellular response detected in our injectable systems [6-8].

Carbon-based nanomaterials displayed a distinct response pattern characterized by acceptable viability at lower concentrations and a gradual reduction in cellular metabolic activity with increased exposure. In our experiments, this dose-dependent behavior was

accompanied by variable adhesion and heterogeneous cell morphology, suggesting a narrow optimization window for these materials. Previous studies have highlighted the potential of carbon nanotubes and graphene-based platforms to improve electrical coupling and cardiomyocyte performance in vitro [10-12]. However, our findings support the notion that careful control of nanomaterial concentration and dispersion is essential to balance bioactivity and cytocompatibility, particularly when integrated into complex cardiac constructs [12,13].

Bioactive biomaterials functionalized with growth factors exhibited the most favorable overall cellular response in our study, with high viability, enhanced adhesion, and increased proliferation. These results underline the importance of biochemical signaling in modulating cellular behavior beyond passive structural support. Collagen-based and hydrogel systems incorporating growth factors have previously demonstrated improved cellular integration and functional outcomes relevant to myocardial repair [13,15]. Our observations further suggest that bioactive functionalization can amplify cellular responsiveness even under simplified in vitro conditions, reinforcing its translational relevance. Additionally, recent advances in biomaterial design incorporating microstructural guidance, piezoelectric properties, or conductive features have emphasized the synergistic role of mechanical and electrical cues in cardiac tissue engineering, aspects that may further potentiate bioactive systems [2,16-18].

Conclusions

Our present in vitro study demonstrates that the composition and functionalization of biomaterials play a decisive role in modulating cell viability, adhesion, morphology, and functional response in heart-relevant cell cultures. Our findings highlight distinct material-dependent cellular behaviors in polymer scaffolding, injectable hydrogels, carbon-based nanomaterials, and bioactive systems. These results underscore the importance of rational selection and optimization of biomaterials in early-stage cardiac tissue engineering, providing a valuable experimental framework for future translational and preclinical investigations.

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Received: December 20, 2025
Accepted: February 19, 2026