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# Functionalized nanoparticles provide early cardioprotection after acute myocardial infarction

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ABSTRACT

Recent developments in nanotechnology have created considerable potential toward diagnosis and cancer therapy. In contrast, the use of nanotechnology in tissue repair or regeneration remains largely unexplored. We hypothesized that intramyocardial injection of insulin-like growth factor (IGF)-1-complexed poly(D,L-lactide-*co*-glycolide) (PLGA) nanoparticles (PLGA-IGF-1 NPs) increases IGF-1 retention, induces Akt phosphorylation, and provides early cardioprotection after acute myocardial infarction (MI). We synthesized 3 different sizes of PLGA particles (60 nm, 200 nm, and 1 μm) which were complexed with IGF-1 using electrostatic force to preserve the biological function of IGF-1. Afterward, we injected PLGA-IGF-1 NPs in the heart after MI directly. Compared with the other two larger particles, the 60 nm-sized PLGA-IGF-1 NPs carried more IGF-1 and induced more Akt phosphorylation in cultured cardiomyocytes. PLGA-IGF-1 NPs also prolonged Akt activation in cardiomyocytes up to 24 h and prevented cardiomyocyte apoptosis induced by doxorubicin in a dose-dependent manner. *In vivo*, PLGA-IGF-1 NP treatment significantly retained more IGF-1 in the myocardium than the IGF-1 alone treatment at 2, 6, 8, and 24 h. Akt phosphorylation was detected in cardiomyocytes 24 h post-MI only in hearts receiving PLGA-IGF-1 NP treatment, but not in hearts receiving injection of PBS, IGF-1 or PLGA NPs. Importantly, a single intramyocardial injection of PLGA-IGF-1 NPs was sufficient to prevent cardiomyocyte apoptosis ($P < 0.001$), reduce infarct size ($P < 0.05$), and improve left ventricle ejection fraction ($P < 0.01$) 21 days after experimental MI in mice. Our results not only demonstrate the potential of nanoparticle-based technology as a new approach to treating MI, but also have significant implications for translation of this technology into clinical therapy for ischemic cardiovascular diseases.



## 1. Introduction

Congestive heart failure, which is predominantly caused by coronary artery disease, is a leading cause of death worldwide [1,2]. In current clinical practice, early revascularization followed by palliative medication is the standard course of treatment for patients with acute myocardial infarction (MI) [3,4]. However, the effect of these treatments often falls short of a cure, exerting only symptomatic control without eliminating the disease, and current therapies may only delay the progression of heart failure. This is particularly true once a substantial number of cardiomyocytes have died after an ischemic attack; the heart has a very limited capacity for self-repair and injury often leads to scar formation, ventricular remodeling, chamber dilatation and, ultimately, heart failure [4,5]. Therefore, there remains an imperative but challenging need to develop new therapies that offer an actual cure for myocardial damage.

Insulin-like growth factor-(IGF)-1-dependent signaling pathway, acting through the IGF-1 receptor, has been suggested to be involved in cardiac development [6]. IGF-1 is also critical in regulating myocardial function and it has been shown to promote cardiomyocyte

*Abbreviations:* PLGA, poly(D,L-lactide-*co*-glycolide); IGF-1, insulin-like growth factor-1; NPs, nanoparticles; MI, myocardial infarction; QDs, quantum dots; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; IVSd, inter ventricular septum dimension; LVEF, left ventricle ejection fraction; FS, fractional shortening.

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growth and protect cardiomyocyte survival *in vitro* and *in vivo* [6,7]. Studies have also shown that IGF-1 treatment improves myocardial function after infarction in experimental models and in humans [8,9]. Furthermore, the observed increase in cardiomyocyte apoptosis after MI in IGF-1 knockout mice indicates the crucial role of IGF-1 in cardiomyocyte protection [10]. Interestingly, although IGF-1 can be used to treat both acute and chronic MI, it has also been reported that prolonged IGF-1 overexpression reduces cardiac functional recovery after MI [11]. Therefore, there is a need to develop a mechanism for the accurately controlled release of IGF-1.

In rats, direct intramyocardial injection of cardiomyocyte survival factor reduces cardiomyocyte death 24 h after acute infarction, leading to a reduction in infarct size and recovery of cardiac function after four months [12,13]. These results suggest that early treatment and effective cardioprotection within the first 24 h after MI are key to successful therapy. Previous studies have used different biomaterials to achieve high cell retention rates for enhanced therapeutic effects [14–20]. In contrast, treating acute MI requires a more immediate effect for cardiomyocyte protection to achieve myocardial function preservation. However, previous approaches to delivering cardioprotective factors have mainly relied on catheter or surgical interventions and used hydrogel materials [12–14,20] or microparticles [21] as carriers for these factors. Although these delivery agents may prolong local drug retention for weeks [12,20], the monitoring and control of drug release kinetics remains an issue. Furthermore, unregulated degradation or extended retention of the delivery carriers may cause undesired local and systemic side effects. In contrast, nanotechnology may improve treatment effectiveness because nanomaterials have unique physicochemical properties that enhance their therapeutic efficiency [22–24]. Previous studies have shown that the particle size influenced the clearance and circulation time [25–27]. Depending on the size and homogeneity of the preparations, the retained microparticles may travel all over the body, and have been found to cause embolism, occluding blood flow especially when delivered intravenously [28]. In contrast, NPs are advantageous in this regard due to the small size and are able to pass through blood vessels [29]. Therefore, with the aim of developing a delivery system that is both easily accessible to the infarcted myocardium and retained within the injection site, we evaluated NPs for their ability to carry IGF-1 and exert early cardioprotection after acute infarction.

## 2. Materials and methods

### 2.1. Nanoparticle synthesis and functionalization

Poly(D,L-lactide-*co*-glycolide) (PLGA, M.W. 35,000–65,000 Da, Bio Invigor Corporation, Taipei, Taiwan) with a 50/50 ratio of lactide to glycolide, was purchased. PLGA was dissolved in 5 ml acetone to a final concentration of 10 mg/ml. An ethanol/$H_2O$ (50/50, % v/v) solution was added dropwise (1 ml/min) to the PLGA solution using a syringe pump and stirred until turbid. After an additional 5 min of stirring, the suspension was added to 20 ml of 1 mM polyethylenimine (PEI, Sigma, St. Louis, MO, USA) in a glass beaker and homogenized at low speed (14 ×*g*) for 20 min. The solution was then filtered through a 0.22 μm filter membrane. The different sizes (60 nm, 200 nm and 1 μm) of PLGA nanoparticles were collected following centrifugation at different speeds (366 ×*g* for 10 min for 1 μm, 5867 ×*g* for 20 min for 200 nm and 17,968 ×*g* for 20 min for 60 nm nanoparticles). The PLGA NPs were washed three times with deionized water. To complex IGF-1 onto the PLGA NPs, the NPs were resuspended in deionized water at pH 10 after the washing steps. Various concentrations (3, 15, 30, 60 and 300 μg/ml) of human IGF-1 (Pepro Tech, Rocky Hill, NJ, USA) were added to the stock PLGA NP solution (5 mg/ml) to constitute different nanoparticle solutions. These cocktail solutions were incubated at 4 °C in a refrigerator for 1 h. The entire synthesis of IGF-1-modified PLGA NPs was conducted on ice. After incubation, these cocktail solutions were centrifuged at 17,968 ×*g* for 20 min and washed three times with deionized water (pH 7). All the supernatants were collected and analyzed using a human-specific IGF-1 ELISA kit (Assaypro, St. Charles, MO, USA) to determine the binding capacity of the products prepared under each set of conditions.

### 2.2. Synthesis of PLGA-QD NPs and characterization of PLGA-IGF-1 NPs

To determine the *in vivo* distribution of PLGA NPs after injection into the infarcted area, we conjugated quantum dots (QDs, Invitrogen, Carlsbad, CA, USA) to the surface of PLGA NPs. The QD-COOH functional groups were linked to the $NH_2$-terminal groups of PLGA NPs by adding 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, Sigma). Already being widely used in biomedical imaging, QDs were used to track the PLGA NPs. Although QDs are shown to be toxic [30], these particles were not used in the actual treatments of PLGA-IGF-1 injections. The surface morphologies of the PLGA NPs, PLGA-IGF-1-complexed NPs and PLGA-QD NPs by negative staining (phosphatidic acid, Sigma) examined by transmission electronic microscopy (TEM, Hitachi 7500). The surface potential of NPs was determined as their zeta potential and total volume 1 ml to examine by dynamic light scattering (Zetasizer ZS, Malvern Industries, Worcestershire, UK) at room temperature. The concentration of IGF-1 was measured using a human-specific IGF-1 ELISA kit (Assaypro).

### 2.3. Experimental myocardial infarction

All animal procedures were approved by the National Cheng Kung University Institutional Animal Care and Use Committee. We used acute MI in mice with immediate injection of treatment as the model. Animals were sacrificed at 3 weeks after MI. Previous studies have shown that after MI, scar formation is complete within 3 weeks [31]. MI was induced in male FVB mice weighing approximately 25 g. Briefly, mice were anesthetized with Zoletil (12.5 mg/kg; Virbac, Carros, France) and Rompun (0.2 ml/kg; Bayer Healthcare, Kiel, Germany). After tracheal intubation, the heart was exposed *via* a left thoracotomy. The left coronary artery was identified after pericardiotomy and ligated by suturing with 6-0 prolene below the left atrial appendix. For the sham operation, suturing was performed without ligation. A total of 20 μl PBS only, IGF-1 only, PLGA only, or PLGA-IGF-1 solution was injected into peri-infarcted area at three sites by insulin syringe (TERUMO U-100, 30G*3/8, Elkton, MD, USA) (equal amount in each injection, $n \geq 8$ per group) immediately after coronary artery ligation. The total dosage of IGF-1 used in both the IGF-1 only and PLGA-IGF-1 groups was 20 ng. After injection, the chest was closed, and the animals were allowed to recover under a heating pad. All experiments were performed blinded.

### 2.4. Statistical analysis

Results are presented as means ± SEM. Statistical comparison was performed with two-tailed Student's *t* tests or one-way or two-way ANOVA. A probability value of $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Synthesis of IGF-1-complexed PLGA nanoparticles

PLGA is a copolymer that has been used as drugs and previously been approved by the FDA for use as medicine. It has been formulated into nanoparticles for biomedical applications because of its low toxicity, high biocompatibility, and efficient biodegradability [32–34]. To prevent protein structural changes in IGF-1, we opted to complex PLGA NPs with IGF-1 using electrostatic force. However, PLGA is negatively charged under physiological conditions (*i.e.* a solution pH of 7.4), as the isoelectric