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The Effect of Gold Nanorods on Cell-Mediated Collagen Remodeling

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Abstract

Cardiac fibroblasts, the non-contractile cells of the heart, contribute to myocardial maintenance through the deposition, degradation, and organization of collagen. Adding polyelectrolyte-coated gold nanorods to 3D constructs composed of collagen and cardiac fibroblasts reduced contraction and altered the expression of mRNAs encoding β -actin, α -smooth muscle actin, and collagen type I. These data show that nanomaterials can modulate cell-mediated matrix remodeling and suggest that the targeted delivery of nanomaterials can be applied for anti-fibrotic therapies.

Gold nanomaterials have received considerable attention due to unique optical properties^{1–3} which make them ideal platforms for biomedical research in biosensing¹, cell tracking⁴, imaging^{5,6}, drug delivery⁷, and therapeutics.⁸ For biomedical applications, a fundamental understanding of how cells interact with and respond to an environment containing gold nanomaterials is crucial. There have been multiple studies documenting the cellular uptake and cytotoxicity of gold nanoparticles in different cell types,^{9–11} but very little is known about how nanoparticles affect cellular functions or interactions between the cells and their extracellular matrix (ECM). Gold nanorods have been used as pattern markers to measure neonatal cardiac fibroblast-induced strain fields in a two-dimensional collagen matrix.⁵ However, cells on a two-dimensional substrate behave differently than in a three-dimensional, tissue-like environment.^{12,13}

To determine if gold nanorods alter the behavior of cardiac fibroblasts in a more *in vivo* like environment, the ability of neonatal rat cardiac fibroblasts to remodel three-dimensional type I collagen gels was examined. Fibroblasts account for ~30% of the cells in the neonatal rat heart,¹⁴ and their primary function is to regulate the abundance and organization of extracellular matrix proteins, including type I collagen. Collagen production increases dramatically in the neonatal heart,¹⁵ an adaptation to increased mechanical load, and is organized into a network similar to that observed in the adult heart.¹⁶ Three-dimensional collagen gels are good *in vitro* models of tissue remodeling,¹² and have been used extensively to examine factors influencing cardiac fibroblast matrix remodeling.^{17,18} During the process of tissue remodeling, a fibroblast undergoes a phenotypic switch to a myofibroblast, characterized by up-regulation of α -smooth muscle actin and increased production of ECM proteins.¹⁹ Myofibroblasts reorganize the ECM by attaching and pulling on the collagen-rich matrix and undergo apoptosis when the remodeling process is complete.²⁰

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To examine the effects of gold nanorods on cell-mediated matrix remodeling, varying amounts of polymer-coated gold nanorods were added to a solution of neutralized collagen, which was then seeded with 200,000 neonatal rat cardiac fibroblasts. Gold nanorods (392 ± 93 nm long, 22 ± 3 nm wide) were prepared in aqueous solution using a seed-mediated surfactant-directed approach previously described and purified by centrifugation and washing.^{21–23} Using layer-by-layer (LbL) assembly²⁴, three layers of oppositely charged polyelectrolytes [poly (diallyldimethylammonium chloride), PDADMAC; poly(styrene sulfonate), PSS] were deposited on the surface of the nanorods terminating in PSS. The polymer coating was used to render the nanorods anionic in aqueous solution, to mask the surfactant bilayer, and reduce potential toxicity. Nanorod-collagen-fibroblasts composite gels were prepared by adding 100 μ L of a solution containing 100 μ L 0.2N HEPES buffer (pH 9.0), 100 μ L 10X MEM, 800 μ L type I collagen, 200,000 neonatal rat cardiac fibroblasts, and varying concentrations of gold nanorods (maximum concentration ~ 20 pM) to a 96 well cell culture plate. The nanorod-collagen-fibroblasts composite gels were allowed to polymerize for 1 h at 5 % CO₂ and 37 °C, detached from the surface of the culture plate, and the contraction of these gels monitored over a 24 hour time period. The addition of polymer-coated nanorods to cardiac fibroblast seeded collagen gels resulted in a significant ($p < 0.05$) dose-dependent inhibition of fibroblast-mediated contraction (Figure 1). The highest concentration of nanorods (5.6×10^9 nanorods/gel) inhibited gel contraction by approximately 10% compared to control gels without nanorods. In addition to impaired contraction, a qualitative difference in the stiffness of collagen gels prepared with nanorods was observed. Control gels lacking nanorods appeared more rigid and better able to hold their shape; in contrast, collagen-nanorod composite gels were more fluid. Transmission electron microscopy showed that within the collagen gels, the nanorods were predominantly associated with the collagen (Figure S1) and in only a few instances were nanorods detected inside the fibroblasts. Turbidity experiments revealed that polymer-coated nanorods reduced the lag time of collagen fibrillogenesis but did not alter the equilibrium of polymerization (data not shown). Confocal reflectance microscopy (Figure 2) showed qualitatively similar collagen distribution within the gels, suggesting the nanorods do not substantially alter collagen organization.

The reduced gel contraction observed in the presence of gold nanorods was not due to cell death or a decrease in cellular ability to degrade the ECM, as measured by a number of assays. A fluorescence cell viability assay showed no difference in fibroblast survival ($\sim 86\%$ viability) between control and nanorod-treated gels (Figure 3). In addition, lactate dehydrogenase release assays indicated that there was no difference in cell necrosis between gels with and without nanorods (Figure S2). Taken together, these results suggest that the observed decrease in gel contraction was not due to nanorod cytotoxicity. Gelatin zymography was used to examine the level of matrix metalloproteinase activity in conditioned media from contracted collagen gels. Previous work has shown that during gel contraction, cardiac fibroblasts produce matrix metalloproteinase 2 (MMP2) and that this enzyme is crucial for the contraction and remodeling process.²⁵ No significant differences in MMP2 activity were detected between control and nanorod-containing constructs (Figure S3), suggesting that the fibroblasts' ability to degrade the ECM was not significantly affected by the presence of the nanorods.

The transition of fibroblasts to a myofibroblast phenotype is a critical event in the remodeling process.¹⁹ A hallmark of this transition is an increase in the expression of α -smooth muscle actin (α -sma) and an increased contractility.¹⁹ Quantitative real-time PCR was carried out on RNA isolated from collagen gels after 24 hours to determine if there was a change in the fibroblast phenotype. In addition to measuring the expression of α -smooth muscle actin, the changes in expression of type I collagen and β -actin mRNAs were also measured. Collagen expression is expected to increase during remodeling, and previous work has shown that β -actin is increased in non-contractile regions of cells.²⁶ A dramatic difference in the expression of these genes was detected in cardiac fibroblasts cultured in collagen gels with and without

gold nanorods (Figure 4). Collagen-nanorod constructs exhibited a significant 447 percent increase in β -actin gene expression compared to control gels ($p < 0.001$; Figure 4). The levels of mRNA coding for both α -smooth muscle actin and type I collagen were significantly ($p < 0.001$) down-regulated in the presence of gold nanorods, by 93 and 69 percent respectively, indicating that there was less α -smooth muscle actin and collagen I expression in gels containing gold nanorods as compared to control gels (Figure 4). Based on this evidence, polymer coated nanorods induced changes in gene expression in cardiac fibroblasts within collagen gels and interfered with the transition of the fibroblasts to a more contractile myofibroblast phenotype.

With the growth of the nanotechnology field, there is increasing interest in characterizing and understanding the effects that nanoparticles have on biological systems with the goal of ultimately using these particles for biomedical imaging, diagnostics and therapeutic approaches. Previous studies have shown that nontoxic gold nanoparticles can be made and used for biomedical applications.²⁷ Nanoparticles have been used for imaging cell-induced changes in the local mechanical environment⁴ and for identification of tumor cells.²⁸ By exploiting the photothermal properties of nanomaterials, these particles have been used to selectively target and destroy tumor cells.⁸ At the cellular level, a size-dependent response has been reported for treatment of cancer cells with functionalized nanoparticles, resulting in changes in both protein levels and cell apoptosis.^{11,29,30} What is unclear is how the responses measured to date will translate into cell behavior on a three-dimensional, tissue scale.

The results of this study offer insight into the cellular response of cardiac fibroblasts to gold nanorods on a tissue-like scale. Related work with lung fibroblasts has shown that ultrafine carbon black particles (~ 14 nm in diameter), but not fine particles (~ 260 nm in diameter), also reduced cell-mediated 3D collagen gel contraction.³¹ This effect was attributed, in part, to adsorption and sequestration of factors known to regulate matrix remodeling, including transforming growth factor β (TGF- β) and fibronectin.³¹ It has also been shown that carboxylated single-walled carbon nanotubes delay, but do not inhibit, collagen gel compaction by aortic smooth muscle cells with minimal cytotoxicity and little effect on cell morphology, but the mechanism remains unclear.³² In our studies, polyelectrolyte-coated gold nanorods, in the size range of the fine carbon black particles, substantially altered the matrix-remodeling behavior of cardiac fibroblasts by interfering with the transition of these cells into myofibroblasts, highly contractile cells which produce collagen and other extracellular matrix proteins as part of a normal wound healing response. Due to differences in cell type and nanomaterial preparations, it is difficult to compare our results with previous reports. Taken together, however, these studies reveal several nanomaterial-induced effects that likely depend on particle size, morphology, and surface chemistry and require further investigation.

Although initially beneficial, continued myofibroblast activity can result in excessive collagen deposition (fibrosis) contributing to progressive organ dysfunction. Myofibroblasts are not found within normal myocardium, but arise in response to pathological stimuli and play a pivotal role in the fibrotic response associated with decreased cardiac function observed in hypertrophic and dilated cardiomyopathies.³³ Currently, few therapeutic approaches exist which specifically target the synthetic activity and transition of fibroblasts into myofibroblasts. The ability to modulate gene expression and fibroblast phenotype suggests that nanoparticles could serve as novel therapeutic agents in regulation of cardiac fibrosis and wound healing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Gold nanorods inhibit fibroblast-mediated collagen gel contraction

A. Photograph of fibroblast-seeded collagen gel without gold nanorods (diameter ~6.5 mm, in a standard 96 well plate) at time zero. B. The same gel from A after 24 hr. C. Photograph of fibroblast-seeded collagen gel with gold nanorods (which provide the color) at time zero. D. The same gel from C after 24 hr. E. Percent change in gel diameter as a function of time. (open square – 2 mg/mL Type I Collagen with 0 nanorods/mL, black square – 1.4×10^9 nanorods/gel, open diamond – 2.8×10^9 nanorods/gel, black diamond – 5.6×10^9 nanorods/gel)

Figure 2. Confocal reflectance images of the collagen microstructure in control (A) and nanorod-containing (B) constructs. Images were collected at identical magnifications. Scale bar = 25 μm .

Figure 3. (Top). Representative confocal images of cardiac fibroblasts in collagen gels with no rods (left) or 5.6×10^9 nanorods/gel (right). Red staining denotes dead cells and green staining denotes living cells. Scale bar = 100 μ m. (Bottom) Histogram showing results of analysis of confocal images ($n = 800$ cells for each treatment). Student's T test was used to determine significance.

Figure 4.

Real time PCR analysis of cardiac fibroblast gene expression. In the presence of polymer-coated gold nanorods, expression of β-actin was 2.2 ± 0.3 fold higher than controls while expression of α-smooth muscle actin and type I collagen were 3.9 ± 1.3 and 1.7 ± 0.1 fold lower, respectively, compared to gels without nanorods. All differences were statistically significant ($p > 0.001$ using a pair wise fixed reallocation randomization).