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Hyaluronic acid-serum hydrogels rapidly restore metabolism of encapsulated stem cells and promote engraftment

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ABSTRACT

Background: Cell death due to anoikis, necrosis and cell egress from transplantation sites limits functional benefits of cellular cardiomyoplasty. Cell dissociation and suspension, which are a pre-requisite for most cell transplantation studies, lead to depression of cellular metabolism and anoikis, which contribute to low engraftment.
Objective: We tissue engineered scaffolds with the goal of rapidly restoring metabolism, promoting viability, proliferation and engraftment of encapsulated stem cells.
Methods: The carboxyl groups of HA were functionalized with N-hydroxysuccinimide (NHS) to yield HA succinimidyl succinate (HA-NHS) groups that react with free amine groups to form amide bonds. HA-NHS was cross-linked by serum to generate HA:Serum (HA:Ser) hydrogels. Physical properties of HA:Ser hydrogels were measured. Effect of encapsulating cardiosphere-derived cells (CDCs) in HA:Ser hydrogels on viability, proliferation, glucose uptake and metabolism was assessed in vitro. In vivo acute intra-myocardial cell retention of ^{18}FDG-labeled CDCs encapsulated in HA:Ser hydrogels was quantified. Effect of CDC encapsulation in HA:Ser hydrogels on in vivo metabolism and engraftment at 7 days was assessed by serial, dual isotope SPECT-CT and bioluminescence imaging of CDCs expressing the Na-iodide symporter and firefly luciferase genes respectively. Effect of HA:Ser hydrogels ± CDCs on cardiac function was assessed at 7 days & 28 days post-infarct.
Results: HA:Ser hydrogels are highly bio-adhesive, biodegradable, promote rapid cell adhesion, glucose uptake and restore bioenergetics of encapsulated cells within 1 h of encapsulation, both in vitro and in vivo. These metabolic scaffolds can be applied epicardially as a patch to beating hearts or injected intramyocardially. HA:Ser hydrogels markedly increase acute intramyocardial retention (~6 fold), promote in vivo viability, proliferation, engraftment of encapsulated stem cells and angiogenesis.
Conclusion: HA:Ser hydrogels serve as 'synthetic stem cell niches' that rapidly restore metabolism of encapsulated stem cells, promote stem cell engraftment and angiogenesis. These first ever, tissue engineered metabolic scaffolds hold promise for clinical translation in conjunction with CDCs and possibly other stem cell types.

1. Introduction

Stem cell therapy offers the promise of organ repair on demand. Experimental and clinical studies indicate small improvements in heart function, which are often not sustained over the long run. An important obstacle to sustained functional benefits is very low levels of acute stem cell retention and engraftment. We have previously demonstrated [1] using cardiosphere-derived cells (CDCs) [2] that dissociation leads to rapid depression of stem cell

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bioenergetics, indicating a relationship between cellular metabolism and adhesion. Molecular imaging studies using micro-PET/CT of ex vivo ^{18}FDG-labeled CDCs reveal that ~80% of injected CDCs are lost into the lungs and systemic circulation in the first hour following intra-myocardial transplantation [3]. These results have motivated us to design scaffolds that boost acute myocardial retention and promote rapid restoration of transplanted cell bioenergetics.

Currently, most clinical and experimental cell therapy protocols in the heart utilize direct injection of isolated cells, resulting in low levels of acute myocardial retention as well as massive cell death (anoikis, necrosis) due to lack of cell–cell or cell-ECM (extracellular matrix) contact [4], and lack of substrates. We have designed autologous, biodegradable, bio-adhesive, hydrophilic scaffolds (hydrogels) that combine serum and hyaluronic acid (HA). We chose serum (which is an important component of cell culture medium) because it can provide substrates/growth factors needed for stem cell survival/proliferation. Furthermore, immobilization of serum in hydrogels can provide RGD (Arg-Gly-Asp) motifs in vitronectin and fibronectin (which are components of serum) [5,6] for cell adhesion (integrin activation) [7]. HA is one of the chief components of cardiac extracellular matrix, has been demonstrated to provide a microenvironment for self-renewal, differentiation of stem cells [8] and is implicated in cell adhesion and motility [8–10]. The degradation products of HA also promote angiogenesis [8], which could promote transplanted cell engraftment and cardiac regeneration.

In this study, the carboxyl groups of HA were modified with N-hydroxysuccinimide (NHS) to yield HA-NHS groups which react with free amine groups present on serum proteins and tissue to form amide bonds, resulting in hydrogels that encapsulate stem cells and adhere to transplanted tissue. HA:Ser hydrogels polymerize when HA-NHS and serum are mixed, can be applied epicardially (as a patch) to beating hearts or delivered by intra-myocardial injection with high levels of acute retention (70–100%). Our novel result is that HA:Ser hydrogels promote restoration of cellular bioenergetics within 1 h of encapsulation, both in vitro and in vivo by promoting rapid cell adhesion.

2. Materials and methods

Modification of Hyaluronic Acid: Chemical modification of carboxyl groups in hyaluronic acid (HA) to amine-reactive N-hydroxysuccinimide esters was achieved by reacting 10%(w/v) HA (MW 16 kDa; LifeCore Biomedical) with 67%(w/v) 1-ethyl-3-(3-diemthylaminopropyl) carbodiimide (EDC; Sigma) and 25%(w/v) N-hydroxyl succinimide (NHS; Sigma) in phosphate-buffered saline (PBS) as previously described [11,12]. The hydroxamate assay [11] revealed that 36 ± 0.8% of the carboxylic acid groups in HA formed sulfo–NHS–esters. The –NHS groups from HA hydrolyzed within 10 min with amide bond formation between carboxylic acid groups in HA and amine groups.

Hydrogel Synthesis: HA:Ser hydrogels were synthesized by chemical crosslinking of –NHS with amine groups present on serum proteins. Specifically, 10%(w/v) HA-NHS dissolved in PBS or IMDM (containing 25 mM glucose) was mixed with an equal volume of serum (from syngeneic WK rats) in a 1:1 (v/v) ratio, at room temperature for 5 min. We chose a 1:1 (v/v) ratio for serum and HA in order to maximize adhesivity and supply of adhesion motifs/growth factors present in serum. In order to ensure functionality of –NHS groups, hydrogels were synthesized within 5 min of dissolving HA-NHS in PBS. HA:PEG hydrogels were prepared by mixing in a 1:1 (v/v) ratio, 10%(w/v) HA-NHS in PBS and 10%(w/v) PEG-$(NH_2)_6$ in HEPES buffer at room temperature and pH 7–7.4 [11].

For in vitro cell proliferation studies, stem cells were suspended in serum and subsequently mixed with HA-NHS (dissolved in PBS) in a 1:1 (v/v) ratio, and cultured in cell–specific culture medium which ensured availability of optimal concentrations of substrates/growth factors to encapsulated stem cells. For in vivo studies, HA-NHS dissolved in IMDM (Invitrogen) and CDCs suspended in serum were each aspirated into separate sterile 0.5 mL syringes connected by sterile plastic tubing. HA-NHS and serum were mixed immediately prior to intra-myocardial injection or epicardial application. Since IMDM is used to culture CDCs in vitro, IMDM which contains 25 mM glucose was used to dissolve HA-NHS for in vivo studies -this ensured availability of glucose to encapsulated CDCs following transplantation.

Measurement of Physical Properties of HA:Ser hydrogels: Hydrogels were prepared as cylindrical blocks, 5 mm in diameter, with a total volume of 50 or 100 μL containing 1:1 (v/v) ratio of 10%(w/v) HA-NHS in PBS and serum, using caps of microcentrifuge tubes as molds. Mechanical and physical properties of HA:Ser hydrogels were characterized by measuring swelling ratio, gelation time, compressive modulus, degradation rate and protein release [11].

Equilibrium swelling ratio analysis [11]: HA:Ser hydrogels were incubated in PBS overnight in order to measure their wet weight at maximum saturation. They were subsequently transferred to pre-weighed microcentrifuge tubes and lyophilized for 48 h in order to measure dry weight. The ratio of wet to dry weight was determined as the swelling ratio of the hydrogels.

Gelation time analysis [11]: Using a 2–200 μL pipetman, HA-NHS and serum were mixed and pipetted up and down until the solutions could no longer be pipetted. The time at which this happened was designated as the gelation time.

Compressive (Young's) modulus analysis [11]: To measure compressive modulus, hydrogel constructs were placed in between two parallel metal plates on an adjustable stage. The bottom plate was attached to a 250 g loading weight and a force transducer, connected to a computer. The gels were then deformed by 1% height in discrete 20sec intervals until 10% deformation was reached (electroforce 3200 testing instrument, Bose). The best fit slope of the stress–strain curve (4–9% strain) was used to calculate compressive modulus.

Degradation rate [11]: Hydrogels can be degraded by hydrolysis, proteases present in tissue and/or secreted by encapsulated CDCs. Since cells can secrete matrix metalloproteinases and hyaluronidases which could accelerate degradation of hydrogels, studies of hydrogel degradation were performed with and without encapsulated cells. Hydrogel constructs (50 μL) without cells ($n = 3$) and hydrogels containing encapsulated CDCs ($n = 5$) were incubated in culture medium at 37 °C for 12 days; hydrogel dry weights were measured every 4 days. Change in gel dry weight was used to quantify degradation rate.

Protein release from HA:Ser hydrogels: Soluble serum proteins from HA:Ser hydrogels can be released over time. In order to assess protein release, HA:Ser hydrogels (50 μL volume; $n = 3$) were incubated in PBS at 37 °C. Sample aliquots (5–40 μL of PBS solution) were obtained over 20 days and protein concentration was measured using the Bradford assay (BioRad). The total volume of PBS was readjusted to 1 mL after each sampling. Total serum protein concentration was determined from 25 μL of serum suspended in 1 mL PBS (equivalent to the hydrogel) in order to normalize results of protein estimation to the total protein content of serum.

2.1. Stem cells

Cardiosphere-derived cells (CDCs) were used for all in vitro and in vivo studies. CDCs are comprised of mixtures of cell populations [13] that express markers of cardiac progenitor cells (c-kit$^+$/CD90$^-$),