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Cardiac and stem cell-cocooned hybrid microspheres: A multi factorial design approach

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ABSTRACT

Cell therapy is a promising approach for the treatment of patients suffering from myocardial infarction. Most recent therapies involve direct injection of cells into the damaged heart tissue to induce regeneration and help restore its functions, however, anoikis and the harsh environment at the sight of injection limit the therapeutic efficacy of current techniques. Biopolymeric microspheres such as alginate have been widely used for cells encapsulation and delivery for cell therapy applications. However, majority of these techniques are not standardized that is a big challenge for translation into clinically-relevant treatment options. In addition, purely-alginate base microspheres are limited by poor biodegradability and lack of strong interaction between the encapsulated cells and their surrounding alginate matrix. In this work, we have shown that the addition of type I collagen into alginate microspheres, systematically optimized by a multivariate experimental design, improves the biocompatibility of the microspheres towards induced pluripotent stem cells (iPS), cardiomyocytes, and blood outgrowth endothelial cells (BOEC), whilst improving diffusion between outside environment and the inner sphere. The addition of collagen allows for multiple routes for sphere degradation leading to potentially greater control over cell release once delivered. Mathematical models were developed and utilized to effectively evaluate and predict the influence of various factors such as polymer ratios, micronization air flow rate, and air-gap distance on spheres size and shape, which play a key role in cell viability, degradation rate of microspheres, as well as controlled production of the cell cocoons toward clinically-relevant cell therapies for treatment of myocardial infarction.

1. Introduction

Cardiovascular diseases (CVDs), including myocardial infarction (MI), are the leading cause of death in the world today [1]. In 2008, an estimated 7.3 million deaths were attributed directly to coronary heart diseases – approximately 12.5% of total mortality [2]. It is projected that the number of people who will die from CVDs, mainly associated with heart disease and stroke, will increase to about 23.3 million by 2030 and will remain the main cause of mortality [3].

MI arises from the blockage of the coronary artery, leading to cardiomyocyte cell death and the formation of scar tissue mostly within the wall of the left ventricle. This scar tissue, or myocardial infarct, is mechanically weaker and unresponsive, compared to the surrounding live tissue, leading to significant side effects if left untreated including, increased diastolic volume and arrhythmia. Currently, MI is treated in combination of surgical (coronary angioplasty or coronary artery bypass grafting) and pharmacological (antiplatelet agents, cholesterol lowering agents,

Abbreviations: iPS, induced pluripotent stem cells; CVDs, cardiovascular diseases; MI, myocardial infarction; C:A, collagen:alginate; MFD, multivariate factorial design.

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angiotensin-converting enzyme inhibitors, beta-blockers) methods. These treatments however, do not remove or regenerate the infarct itself, and if end stage heart failure is reached either mechanically supportive meshes, or, as a last case scenario, organ transplantation are viable options. The use of stem cell tissue engineering to repair and re-grow the damaged tissue of the infarct provides an exciting alternative avenue for the treatment of MI. Initial studies using direct injection of stem cells onto an infarct site by Amanto et al. [4], Murry et al. [5], and Shake et al. [6], demonstrated the potential efficacy of this approach.

In recent years tissue engineering and regenerative medicine have gained another promising tool for treatment of MI, namely induced pluripotent stem (iPS) cells [7]. iPS cells are generated by introducing specific transcription factors (e.g. *Oct4, Sox2, Klf4* and *c-myc*) into adult somatic cells, which reprogram somatic cells into an undifferentiated, pluripotent state similar to embryonic stem cells. The resulting cells have the ability to differentiate into all three germ layers [8,9]. With this method ethical concerns associated with embryonic stem cells may be avoided, as no oocytes or embryos are used. Furthermore, patient-specific cells can be used, which eliminates the risk of immune rejection and makes them ideal for drug screening, disease modeling, or regenerative medicine [10].

Blood outgrowth endothelial cells (BOEC) have also been reported as good candidates for vascular regenerating cell therapy. It was found that BOEC were able to form vasculature tubular structures in a three-dimensional fibrin matrix comparable to VEGF or bFGF, which is crucial in cardiac therapy. Factor VIII (FVIII) is also known as an essential blood-clotting protein. Elevated levels of FVIII are also known to cause thrombosis within a blood vessel resulting in myocardial infarction [11,12].

Matrix-assisted cell therapy techniques are a focus of intense research worldwide due to the exceptional social and economic impact that such versatile techniques would provide [13–15]. One such matrix assisted technique that has attracted significant attention is the encapsulation of stem cells into biodegradable microspheres [16–18]. However, the impediment in the development of these microspheres has been the lack of appropriate clinical-grade cell-interactive biopolymers able to form microspheres [19] and the degradation rate of such spheres. Homogeneity and reproducibility of the spheres are another obstacle [19]. One material that has gained significant use for encapsulation for MI treatment is sodium alginate due to the ease of cross-linking through ionotropic reaction along with the inherent biocompatibility of alginate [14,16,19–21]. While alginate has been used as a cell encapsulation matrix for a variety of applications including cardiovascular diseases, brain tumours, haemophilia, and diabetes, several limitations to using alginate as a sole material have emerged [14]. Specifically, the alginate matrices have not allowed for cells to leave the matrix and interact with the target site, either through matrix degradation or cellular diffusion through matrix pores [20,22]. Furthermore, for MI specific treatments alginate spheres have demonstrated poor levels of vascularization through the matrix [22]. These limitations have led to a plethora of studies on the combination of alginate and other biopolymers for cell encapsulation, including poly-L-lysine [23], poly-L-ornithine [24], poly(methylene-co-guanidine) [25], poly(ethylene glycol) [26], chitosan [26], cellulose [27], carrageenan [15], and protamine sulfate [28]. However, none of these combinational biomaterials have provided an optimal solution for treating MI.

In contrast to alginate, collagen has a very high cell adhesion propensity, crucially, can be degraded enzymatically within the body, and cannot be easily and rapidly cross-linked iono-tropically. Furthermore, Sato et al. [29] demonstrated that upregulation of collagen synthesis by ascorbic acid enhanced the differentiation embryonic stem cells into cardiomyocytes. This result suggests that collagen may be an ideal component of a matrix material for cardiac tissue engineering. Surprisingly, while Dixon et al. [30] demonstrated the effective combination of collagen and alginate for tissue engineering, Yao et al. [31] and Capone et al. [32] have demonstrated the efficacy of utilizing collagen and alginate microspheres for adipose tissue engineering and cell nurturing respectively.

Here we report such a formation of spheres through the combination of the naturally occurring, clinical grade, collagen (porcine Type I) with alginate. We studied the sphere formation, harboring iPS, cardiomyocytes, and FVIII-secreting BOECs while assessing degradability of these composite materials, cell viability, and cell-released bioactivity of FVIII. A system of multifactorial design and mathematical modeling was used to optimize the microspheres formulations, determine the impacts of multiple factors on size and roundness of spheres simultaneously and to predict their behaviors.

Table 1
Multivariate factorial design table, examining effect of air-flow rate, C:A ratio, and air-gap distance on the size, shape, and homogeneity of the microspheres.

#	Flow Rate	Ratio	Distance	Flow Rate (L/min)	Ratio C:A	Distance (mm)
1	−1	1	1	6	2:1	70
2	−1	1	−1	6	2:1	35
3	−1	−1	1	6	0.5:1	70
4	−1	−1	−1	6	0.5:1	35
5	1	1	1	12	2:1	70
6	1	1	−1	12	2:1	35
7	1	−1	1	12	0.5:1	70
8	1	−1	−1	12	0.5:1	35
9	0	0	0	9	1:1	52.5

2. Methods

2.1. Microsphere synthesis and characterization

Collagen (Porcine Type I) was purchased as a 1% solution and then freeze-dried for storage. Collagen was reconstituted in sterile Milli-Q water to form a 2% w/v solution. Alginate powder was dissolved in sterile Milli-Q to form a 2% w/v solution. The 2% w/v collagen and alginate solutions were mixed in a sterile 5 mL plastic syringe at ratios Collagen:Alginate (C:A) 0.5:1, 1:1, and 2:1. The encapsulation of BOECs was performed by adding 100 μL of BOEC cell suspension (~700,000 cells/mL) to 1 mL of the collagen-alginate mixtures and dispensed in a drop-wise manner in a 2% $CaCl_2$ bath while being stirred. No mechanical or external source of energy was used to micronize the mixtures. As for other cell lines, microsphere synthesis experiments were conducted using an air jet micronization system and experiments were performed according to a 2^3 multivariate factorial design (MFD) examining the effect of air-flow rate, C:A ratio and air-gap distance on the produced size, shape and homogeneity of the microspheres. The air-gap distance was defined as the distance the polymer droplets travelled in the air from the tip of the nozzle to the point where they hit the surface of the crosslinking solution. The parameters used for this multivariate experimental design were analyzed using Minitab 14 software as summarized in Table 1. To mathematically describe the magnitude of the effects of factors on responses, each response data set was fitted with a standard form of a 3rd order linear regression model:

$$R_n = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (1)$$

where R_n represents the response, while X_1, X_2, and X_3 indicate the key factors. X_1X_2, X_1X_3 and X_2X_3 denote the two-way interactions, and $X_1X_2X_3$ denotes the three-way interaction. β_0 is the coefficient for constant term while β_1 *to* β_{123} stand for the effect coefficients associated with the individual factors and their interactions.