EVs–PEG–cECMH Product

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The combination of cardiosphere-derived extracellular vesciles (EVs), polyethylene glycol (PEG), and cardiac extracellular matrix hydrogel (cECMH), EVs–PEG–cECMH, is a potential multipronged product with improved gelation time and mechanical properties, increased on-site retention, and maintained bioactivity that, all together, may translate into boosted therapeutic efficacy.

Keywords: extracellular vesicles ; hydrogel ; extracellular matrix ; polyethylene glycol

1. Introduction

Cardiovascular disease (CVD) is a class of diseases that involve the heart or blood vessels [1]. CVD includes coronary artery diseases such as angina and myocardial infarction (commonly known as a heart attack) and some ageing related pathologies. The underlying mechanisms vary deep ending on the disease, but as the adult heart presents limited ability to self-repair and regenerate, it becomes necessary to explore new biological therapies that are able to prevent or revert these pathological changes [2].

In recent years, there are many methods and products being studied for curing CVD. However, regenerative and reparative products present demanding requirements that are difficult to achieve and that limit their translation into the clinical scenario [3]. They must be biocompatible and bioactive, preferably injectable in a unique dose, and adequately retained and degraded at the site of interest.

The main problems of cell products derive from their large variability and their lack of stability and standardization[4]. Some of the challenges seemed to be solved once it was clarified that most of their beneficial effects are caused by paracrine mediators, such as extracellular vesicles (EVs), which possess meaningful advantages as therapeutics vs. their parenteral cells [5][6]. EVs have been shown to halt proinflammatory and profibrotic pathways and induce angiogenesis [7]. However, EV retention at the target site remains as one of their main challenges [8].

The use of injectable and natural biomaterials for cardiac regeneration also seems promising, but they still present some limitations. For example, extracellular matrix hydrogels provide mechanical support and an environment with a structure and protein composition close to the native cardiac tissue[9], but have slow gelation time, rapid degradation, and poor mechanical properties[10]. The combination of naturally derived hydrogels with synthetic materials, such as polyethylene glycol (PEG), seems promising[10] to make cECMH more suitable for therapeutic use. Apart from providing structural support and favorable bioactivity on surrounding tissue, biomaterials can also be used for the delivery of small particles and/or cells to improve their retention at the injection site[11].

The combination of hydrogels with other bioactive products (EVs–PEG–cECMH) may solve some of the current limitations they possess individually and enhance the therapeutic response with a synergistic effect[12].

2. Mechanism

cECMH have collagen as their main component, so they share some similarities in the gelation kinetics with collagen hydrogels[13]. During the gelation process, first collagen nucleation occurs by forming triple helices. Later, these assemble into ordered structures to form fibrils[14]. The addition of linear, low molecular weight PEG seems to speed up the nucleation phase and favor collagen fibril formation during gelation. This more rapid formation could be responsible for the larger fiber diameter[15], the higher turbidity of the gels[14] and the fast gelation time of the combined product. While other studies have incorporated PEG into cECM or collagen hydrogels to tailor their material properties[15][16], these studies incorporate multi-armed PEG with functionalized groups that crosslink with the collagen network or modified PEGs that require external triggers for polymerization, such as UV light. The addition of functionalized multi-armed PEGs at high

concentrations still allows the formation of gels at 37 °C without external triggers and the tuning of mechanical properties and degradation, but differently to the method presented here, they do not improve the gelation time[15]. Slow gelation times, which can increase tissue necrosis, have been highlighted as a main draw-back of ECM hydrogels[10].

The fast gelation time of the combined product probably prevents the rapid absorption of the subcutaneously injected EVs into the bloodstream right after the injection, as EVs administered in vivo in the PEG–cECM solution were better retained at the target site when compared to EVs administered with the standard vehicle (PBS).

Mechanical properties of the ECM hydrogels are also improved with the incorporation of PEG, while injectability and biodegradation are maintained. The mechanical properties of cECMH alone are considered insufficiently robust for providing prolonged me-chanical support in the injured heart, where they are subjected to significant strain and contraction[10]. In addition, these properties influence cell fate[17] and migration[18]. PEG–cECMH or EVs–PEG–cECMH present a significantly higher storage modulus, making them more suitable for cardiac applications than cECMH alone. The reduction in the gelation time and the increase in the elasticity of the cECMH after gelation did not negatively affect the viscosity of the liquid form and their injectability through the MyoStar catheter. Even the solutions with reduced gelation time could be uniformly injected and did not clog the catheter, an essential property for cardiac applications[19]. In fact, when adding the EVs, the force required for injection was considerably lower. Moreover, the incorporation of PEG with this method does not influence the cECMH biodegradation rate, which in vivo studies have shown to completely degrade within 14–28 days post-injection[9].

3. Medical Uses

The combination of EVs–PEG–cECMH optimizes the features of its individual bioactive components and makes it more suitable and effective in different therapeutical applications. EVs–PEG–cECMH maintained or significantly improved the physicochemical properties (particularly the gelation time), while not hindering injectability and degradation vs. cECMH alone. PEG at low concentrations, which, in fact, can be used to isolate EVs from conditioned medium, was responsible for these differences. In addition, the EVs are progressively released from the EVs–PEG–cECMH and are better retained at the injection site in vivo when administered in the EVs–PEG–cECMH compared to EVs administered in the standard vehicle (PBS). The combination of the products reduces cellular senescence, maintaining the bioactive properties of cECMH and EVs alone. These improved properties of the combined product solve some of the current limitations of the individual use of these regenerative components, which may be translated into an increased therapeutic efficacy.

References

1. extracellular vesicles;hydrogel;extracellular matrix;polyethylene glycol
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