ARTIGO

SODIUM ALGINATE/PRP MATRICES FOR PROSTAGLANDIN RELEASE IN ACUTE MYOCARDIAL INFARCTION

MATRIZES DE ALGINATO DE SÓDIO/PRP PARA LIBERAÇÃO DE PROSTAGLANDINA NO INFARTO AGUDO DO MIOCÁRDIO

DOI: 10.56083/RCV4N1-023
Recebimento do original: 01/12/2023
Aceitação para publicação: 05/01/2024

Carlos de Almeida Barbosa
PhD in Health Technology
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba - PR, CEP: 80215-901
E-mail: carlos.b@pucpr.edu.br

Rossana Baggio Simeoni
Postdoctoral Student in Health Sciences
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba - PR, CEP: 80215-901
E-mail: rosanabagio@gmail.com

Maria Fernanda Villaça Koh
Master in Food Security and Nutrition
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba - PR, CEP: 80215-901
E-mail: mariafernandatemporal@gmail.com

Luize Kremer Gamba
Master's Degree in Health Sciences
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba - PR, CEP: 80215-901
E-mail: mariafernandatemporal@gmail.com
Marcos Antonio Denk  
Bachelor in Biomedicine  
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)  
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba -PR, CEP: 80215-901  
E-mail: slidesdenk@gmail.com

Júlio Cesar Francisco  
Post-Doctor Health Sciences  
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)  
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba -PR, CEP: 80215-901  
E-mail: julio.apfr@gmail.com

Luiz César Guarita-Souza  
Post-Doctor by Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo  
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)  
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba -PR, CEP: 80215-901  
E-mail: luiz.souza@pucpr.edu.br

Beatriz Luci Fernandes  
Post doctorate in Biomedical Engineering by Universidade Tecnológica Federal do Paraná (UTFPR)  
Institution: Pontifícia Universidade Católica do Paraná (PUC-PR)  
Address: R. Imaculada Conceição, 1155, Prado Velho, Curitiba -PR, CEP: 80215-901  
E-mail: beatriz.fernandes@pucpr.edu.br

ABSTRACT: Tissue engineering is a multidisciplinary field, and biocompatibility, biodegradability, shear behavior, rapid gelation, and an easy cross-linking process make alginate one of the most extensively studied polysaccharides in this area. Myocardial infarction represents the leading cause of death worldwide. Recently, peroxisome proliferator-activated receptors (PPARs) have garnered research interest due to their role in modulating inflammation. 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) has demonstrated significant antinociceptive and anti-inflammatory activity. In the pursuit of a shorter and less invasive drug delivery system, a biodegradable matrix was sought. Leveraging the characteristics of alginate and platelet-rich plasma (PRP) as biodegradable biomaterials with suitable physicochemical and biological properties for this application, this study aimed to develop an alginate and PRP biomaterial for prostaglandin release. Platelet-Rich Plasma (PRP) was obtained by concentrating platelets through centrifugation. The biomaterial, in the form of membranes, was prepared using the casting method from polymeric solutions with a concentration of 4.0% (w/v), with PRP added in proportions of 10% relative to the mass of sodium alginate (SA). The preformed membranes were immersed in aqueous solutions of 1% CaCl2 (w/v) to promote SA cross-linking and subsequent drug incorporation. In vitro bioac-ivity was evaluated by immersion in simulated body fluid. The SEM assay demonstrated that the incorporation of SA/PRP/PJ occurred uniformly. Therefore, the results suggest the
potential use of alginate and PRP films for prostaglandin release, but pre-clinical studies are still required.

**KEYWORDS:** Chronic Wounds, 3D Printing, Alginate, 15d-PGJ (2), Acute Myocardium Infarction, Release System.

**RESUMO:** A engenharia de tecidos é um campo multidisciplinar, e a biocompatibilidade, a biodegradabilidade, o comportamento de cisalhamento, a rápida gelificação e o fácil processo de ligação cruzada fazem do alginato um dos polissacarídeos mais estudados nessa área. O infarto do miocárdio representa a principal causa de morte em todo o mundo. Recentemente, os receptores ativados por proliferadores de peroxissoma (PPARs) despertaram o interesse da pesquisa devido à sua função na modulação da inflamação. A 15-deoxi-Δ12,14-prostaglandina J2 (15d-PGJ2) demonstrou atividade antinociceptiva e anti-inflamatória significativa. Na busca por um sistema de administração de medicamentos mais curto e menos invasivo, procurou-se uma matriz biodegradável. Aproveitando as características do alginato e do plasma rico em plaquetas (PRP) como biomateriais biodegradáveis com propriedades físico-químicas e biológicas adequadas para essa aplicação, este estudo teve como objetivo desenvolver um biomaterial de alginato e PRP para a liberação de prostaglandina. O plasma rico em plaquetas (PRP) foi obtido pela concentração de plaquetas por meio de centrifugação. O biomaterial, na forma de membranas, foi preparado usando o método de moldagem a partir de soluções poliméricas com uma concentração de 4,0% (p/v), com PRP adicionado em proporções de 10% em relação à massa de alginato de sódio (SA). As membranas pré-formadas foram imersas em soluções aquosas de CaCl2 a 1% (p/v) para promover a ligação cruzada do SA e a subsequente incorporação do medicamento. A bioatividade in vitro foi avaliada pela imersão em fluido corporal simulado. O ensaio de MEV demonstrou que a incorporação de SA/PRP/PGJ ocorreu de maneira uniforme. Portanto, os resultados sugerem o uso potencial de filmes de alginato e PRP para a liberação de prostaglandina, mas ainda são necessários estudos pré-clínicos.

**PALAVRAS-CHAVE:** Feridas Crônicas, Impressão 3D, Alginato, 15d-PGJ (2), Infarto Agudo do Miocárdio, Sistema de Liberação.
1. Introduction

Tissue engineering is a field of study that encompasses various areas of knowledge, and biocompatibility, biodegradability, shear behavior, rapid gelation, and an easy cross-linking process make alginate one of the most studied polysaccharides in this area [1]. One of the most daunting challenges in human healthcare involves the restoration of damaged or degenerated tissues and the replacement of failed or-gans, posing significant obstacles for modern medicine [2].

Ischemic heart disease is the most serious public health problem, with 126.5 million people affected worldwide. However, recent treatments for myocardial infarction (MI) are still more palliative than curative [3]. Presently, biomaterial scaffolds with or without stem cells or excipients loaded with growth factors, cytokines thera-pies are considered the most promising alternative treatments in re-generative medicine [4]. Recently, engineered heart tissue use of natural biomaterials such as alginate hydrogel with formulations and 3D printing technology, due to good biocompatibility, for scaffold for myocardial repair [5].

Autologous platelet-rich plasma (PRP) is the fraction of blood that has been processed to contain a higher concentration of platelets than the baseline, and it is rich in a diverse array of growth factors, including primary growth factors (GFs), connecting tissue growth fac-tor (CTGF), epidermal growth factors (EGF), Vascular Endothelial Growth Factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factors (FGFs), transforming growth factor beta (TGF-β), insu-lin-like growth factor-I (IGF-I) and platelet-derived growth factor (PDGF) which can promote repair and regeneration [6]. In this study, we developed a matrix alginate-based composite SA/PRP/PGJ highly promising promote the recovery of cardiac function in the MI.
2. Materials and Methods

2.1 Preparation Of 15D-PGJ2 Loaded PLGA NanopartiCles

The synthesis of 15d-PGJ2-NP followed the protocol detailed in the work of Alves et al. [7]. In this procedure, 100 mg of PLGA polymer was dissolved in 30 mL of acetone, along with 100 g of 15d-PGJ2 (Sigma-Aldrich, St. Louis, MO, USA), 40 mg of Sorbian monos-tearate, and 200 mg of caprylic/capric acid triglyceride, collectively comprising the organic phase. Simultaneously, the aqueous phase was prepared by combining 60 mg of polysorbate 80 with 30 mL of deionized water. Once all components in both phases had completely dissolved, the organic phase was gently merged with the aqueous phase, and the resulting mixture was agitated for 10 minutes. Subsequently, the acetone solvent, in a 60/40 (v/v) ratio, was removed via evaporation. The suspension was then concentrated to a final volume of 10 mL under reduced pressure, utilizing a rotary evapora-tor. This process resulted in a 15d-PGJ2 suspension with a concentra-tion of 10 g/m. Any remaining solvent was evaporated until no trac-es of acetone were detectable in the preparation, a crucial step con-firmed by HPLC analysis, as previously detailed by Napimoga et al. in 2020 [8].

2.2 Preparation of Sodium Alginate (SA)

Preparing (SA) Hydrogel involved the following step. The sodium al-ginate powder (purchased from Sigma-Aldrich, St. Louis, MO, USA) polymeric solution was prepared at a concentration of 4.0% (w/v), The preparation was carried out in a clean and sterilized beaker. Firstly, 1 gram of sodium alginate was meticulously dissolved in 10 mL of deionized water.
and to facilitate the crosslinking of the alginate, a CaCl2 solution (1% w/v) was prepared. The crosslinking was proposed in a 4:1 ratio, respectively.

2.3 Preparation of Platelet Rich Plasma (PRP)

PRP was generated using a double-spin centrifugation procedure (Al-Maawi et al., 2021). Initially, blood was collected into a tube containing sodium citrate. Subsequently, the blood underwent centrifugation at 400g for 10 minutes, and the resulting plasma was transferred to a new tube for a secondary centrifugation at 400g for 10 minutes at 4°C. Following this centrifugation step, 50% of the supernatant (known as platelet-poor plasma or PPP) was removed [9].

2.4 Scanning Electron Microscopy

SEM analysis of SA/PRP samples involved several steps. Firstly, sodium alginate samples were subjected to lyophilization for 24 hours at -60°C using the Heto Power Dry PL3000 lyophilizer. The resulting dried material was then gold-coated and prepared for examination. Subsequently, SEM (Scanning Electron Microscopy) with a Philips XL-30 instrument from Eindhoven, the surface pore sizes and the dimensions of SA/PRP samples were measured through SEM micro-photographs, analyzing between 50 and 110 measurements, employing image visualization software (ZEN lite 3.1, ZEN microscopy software., Germany).

3. Results and Discussion

In general, 3D bioprinting is a dynamic and advancing field that offers creative solutions for tissue engineering. Three-dimensional printed scaffolds
can be infused with cells or platelet and can be di-rectly printed within scaffold polymers. In our research, we directly 3D printed an alginate/PRP bioink [10]. In this research, the SA/PRP/PGJ hybrid scaffold was fabricated through a straightforward and adaptable process. Initially, the solutions of nanoparticle, platelet and sodium alginate were combined.

3.1 Sem Topography Analysis of the ALG/PRP/PGJ

SEM analysis (Figure 1) was conducted to study the morphology of the ALG, and SA/PRP+PGJ samples. It is evident that all the scaffolds were composed of uniform fibers and exhibit a porous microstructure with variations in pore size. Notably, SEM topography revealed the presence of a well-interconnected porous morphology in all samples. The micrographs of the hydrogels containing alginate displayed a more asymmetrical and disorganized, porous structure, while the SA/PRP hydrogel exhibited a fine porous structure with a relatively uniform distribution.

Figure 1: SEM images de SA and SA/PRP/PGJ. (a) pore size SA membrane, (b) pore size of PRP membrane, (c) pore size of PGJ membrane, (d) pore size of SA+PRP+PGJ membrane. Scale bar: 100/200 μm.

Source: The Authors.
Palacio-Mancheno et al., describe that the level of porosity within the scaffold materials is vital for effectively facilitating cell adhesion and growth, and it should align with the natural porosity of human tissue. The results of the porosity measurements are presented in Table 1, demonstrating that the composition of the chemical hydrogel significantly influences porosity [11]. In this study, the obtained trend can be explained by the higher swelling capacity of the hydrogels based on alginate, which possesses a larger content of water, resulting in larger pore formation from ice during the freeze-drying process. Previous studies have reported that the higher density the scaffold is, the lower swelling degree it possesses in agreement with the results of morphology analysis [12].

In a particular study, a 3D-printed model was employed, utilizing a bioink composed of porcine decellularized extracellular matrix (dECM) derived from porcine liver, with varying concentrations of gelatin and sodium alginate. This study revealed that the size of the pores played a beneficial role in enhancing the efficiency of metabolic processes and the exchange of nutrients and waste products. This was attributed to the intricate porous structure of the scaffold, which facilitated cell adhesion and invasion, thereby promoting growth within the scaffold [13].

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average pore size µm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4:1</td>
</tr>
<tr>
<td>SA</td>
<td>52.2± 17</td>
</tr>
<tr>
<td>PRP</td>
<td>51.8± 14</td>
</tr>
<tr>
<td>PJG</td>
<td>52.5± 17</td>
</tr>
<tr>
<td>SA/PRP+PGJ</td>
<td>54.9 ± 16</td>
</tr>
</tbody>
</table>

Source: The Authors.

Table 1. The average pore size of SA and SA/PRP/PGJ scaffolds
4. Conclusion

This study introduces a novel hydrogel-based delivery system (SA/PRP/PGJ) with promising attributes and excellent biodegradability and biocompatibility. The findings from this research offer new avenues for advancing myocardial infarction repair strategies.
References


7. ALVES C, DE MELO N, FRACETO L, DE ARAÚJO D, NAPIMOGA M. EFFECTS OF 15D-PGJ2-LOADED POLY(D,L-LACTIDE-CO-GLYCOLIDE)


