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# Hydrogel elasticity and microarchitecture regulate dental-derived mesenchymal stem cell-host immune system cross-talk

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ABSTRACT

The host immune system (T-lymphocytes and their pro-inflammatory cytokines) has been shown to compromise bone regeneration ability of mesenchymal stem cells (MSCs). We have recently shown that hydrogel, used as an encapsulating biomaterial affects the cross-talk among host immune cells and MSCs. However, the role of hydrogel elasticity and porosity in regulation of cross-talk between dental-derived MSCs and immune cells is unclear. In this study, we demonstrate that the modulus of elasticity and porosity of the scaffold influence T-lymphocyte-dental MSC interplay by regulating the penetration of inflammatory T cells and their cytokines. Moreover, we demonstrated that alginate hydrogels with different elasticity and microporous structure can regulate the viability and determine the fate of the encapsulated MSCs through modulation of NF-kB pathway. Our *in vivo* data show that alginate hydrogels with smaller pores and higher elasticity could prevent pro-inflammatory cytokine-induced MSC apoptosis by down-regulating the Caspase-3- and 8- associated proapoptotic cascades, leading to higher amounts of ectopic bone regeneration. Additionally, dental-derived MSCs encapsulated in hydrogel with higher elasticity exhibited lower expression levels of NF-kB p65 and Cox-2 *in vivo*. Taken together, our findings demonstrate that the mechanical characteristics and microarchitecture of the microenvironment encapsulating MSCs, in addition to presence of T-lymphocytes and their pro-inflammatory cytokines, affect the fate of encapsulated dental-derived MSCs.

**Statement of significance**

In this study, we demonstrate that alginate hydrogel regulates the viability and the fate of the encapsulated dental-derived MSCs through modulation of NF-kB pathway. Alginate hydrogels with smaller pores and higher elasticity prevent pro-inflammatory cytokine-induced MSC apoptosis by down-regulating the Caspase-3- and 8- associated proapoptotic cascade, leading to higher amounts of ectopic bone regeneration. MSCs encapsulated in hydrogel with higher elasticity exhibited lower expression levels of NF-kB p65 and Cox-2 *in vivo*. These findings confirm that the fate of encapsulated MSCs are affected by the stiffness and microarchitecture of the encapsulating hydrogel biomaterial, as well as presence of T-lymphocytes/pro-inflammatory cytokines providing evidence concerning material science, stem cell biology, the molecular mechanism of dental-derived MSC-associated therapies, and the potential clinical therapeutic impact of MSCs.



## 1. Introduction

Craniofacial bone tissue engineering currently relies extensively on bone grafting procedures [1–3]. However, there are numerous well-documented drawbacks related to this therapeutic approach [4–7]. Mesenchymal stem cells (MSCs) represent an alternative

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treatment modality in regenerative medicine [4–10]. MSCs derived from orofacial tissues (e.g., stem cells from human exfoliated deciduous teeth (SHED)) are attractive postnatal stem cells with desirable self-renewal capacity and plasticity and osteogenic properties comparable to bone marrow mesenchymal stem cells (BMMSCs) [10–17]. SHED are easily found in pediatric patient's mouth or in tissue waste in pediatric dental clinics. Preclinical and clinical investigations have revealed that SHED are able to generate new bone with great potential for bone repair/regeneration [15,16]. Moreover, these cells are originated from neural crest that makes them particularly compatible for regeneration and repair of neural crest-derived tissues (e.g., jaw bone) [13–19]. Recent studies have emphasized, however, that the cell source is not the sole determinant of success in stem cell-mediated regenerative medicine. The host immune system can have an adverse effect on outcomes. For example, proinflammatory T-cells and cytokines inhibit MSC-mediated bone tissue regeneration [20–22]. These effects can be modulated by local administration of anti-inflammatory agents (e.g. aspirin or indomethacin), which have been shown to improve MSC-mediated bone regeneration [21,22].

The choice of carrier can also strongly affect the performance of MSCs in tissue engineering applications, in part due to immunomodulatory effects, and also because they offer the opportunity to direct the stem cells fate towards the desired phenotype [22–24]. However, regulation of the encapsulated MSCs' is still the main challenge. Hydrogel scaffolds have been largely utilized to study the interaction of biomaterial and MSCs [25–28] RGD-coupled alginate hydrogel has been used extensively for cell encapsulation in bone tissue regeneration [27–35]. Alginate hydrogel can delay the penetration of the inflammatory cytokines and T cells, and act as a physical shield to protect the implanted MSCs from the immune cell attack [22]. Therefore, the scaffold material can affect the cross-talk between MSC and host immune cell, control the implanted MSCs fate, toward improved tissue regeneration quality. Furthermore, it is understood that physiomechanical properties including porosity of the biomaterial influence MSC differentiation, but their roles in the MSC-host immune interaction are fairly unknown [36]. There are no studies evaluating the role of porosity and elasticity of the biomaterial in MSC-proinflammatory T cell/cytokine interplay. How this interplay affects the bone regenerative properties of dental-derived MSCs in particular, has received little attention.

Understanding the factors influencing the fate of encapsulating MSCs is of major therapeutic interest [36]. To develop effective MSC-based regenerative therapies it is crucial to have a clear understanding of how the physiomechanical properties including porosity and elasticity of the encapsulating biomaterial affect the cross-talk between the immune cells and MSCs. Hence, the main goal of our study was to clarify the role of mechanical properties and microarchitecture of the hydrogel biomaterial in directing the fate of encapsulated dental-derived MSCs toward osteogenic tissues.

## 2. Materials and methods

### 2.1. Cell isolation and culture

SHED were used and cultured according to published protocols [15,16] with required IRB approval. The pulp tissues were separated from exfoliated human primary upper and lower central or lateral incisors (isolated from twelve children aged 6–12) and then processed according to methods in the literature [15,16]. Mouse Pan T lymphocytes were isolated according to previously published protocols [21,22] using a magnetic cell sorter (Pan T Cell Isolation Kit II, Miltenyi Biotec, San Diego, CA).

### 2.2. Biomaterial fabrication and cell encapsulation

High molecular weight RGD-modified alginate (NovaMatrix, Norway) was utilized as the encapsulating biomaterial [22,37,38]. The alginate was charcoal treated and oxidized. Subsequently, the hydrogel biomaterials were sterile filtered (0.22 μm filters, Millipore, Billerica, MA) prior to the cell encapsulation process. $2 \times 10^6$ SHED were encapsulated in 1 mL of 1.2% w/v alginate hydrogel. Alginate microspheres were formed employing a microfluidic device and dropwise addition of alginate to $CaCl_2$ solutions with two concentrations: 100 or 200 mM. In order to generate biomaterials with different moduli of elasticity and, therefore, porosity, two different calcium ion concentrations (100 and 200 mM) were used. As the negative group, cell-free alginate hydrogel was utilized.

### 2.3. Characterization of the fabricated hydrogel scaffolds

The Young's moduli of the fabricated alginate hydrogels were analyzed in accordance with previously published methods [39]. The cross-sectional microstructure of the prepared alginate scaffolds were analyzed using scanning electron microscopy (SEM). Lyophilized hydrogels were freeze-fractured (using liquid nitrogen) to expose a cross-section. The scaffold specimens were imaged without further coating using a ZEISS Supra 40VP scanning electron microscope (Zeiss Microscopy GmbH, Jena, Germany). The porosity was further characterized using SEM images by evaluating at least 40 pores using the ImageJ program (National Institutes of Health, Bethesda, MD).

To determine the influence of the elasticity of the hydrogel on its permeability, the release profiles of Trypsin inhibitor (from turkey egg white, 20 kDa; Sigma), bovine serum albumin (BSA, 66 kDa; Sigma), and IgG (from rat serum, 150 kDa; Sigma) from alginate scaffolds were evaluated in PBS solution at different time points. Subsequently, UV spectrophotometer (at 320 nm, Beckman, Brea, CA) was utilized to analyze the amount of released protein for up to two weeks. Additionally, the permeability of the alginate hydrogels to TNF-α (BioLegend, San Diego CA) was evaluated. Briefly, alginate hydrogels were immersed in a 200 μg/mL solution of TNF-α. After 6, 12 and 24 h, the diffused cytokine in the hydrogels was detected using fluorescent microscopy with anti-TNF-α antibodies (Abcam). Seven specimens (n = 7) were analyzed for each group.

### 2.4. SHED-T lymphocyte interaction analysis

To examine the apoptosis of SHED (passage 4) in the presence of T lymphocytes, $0.5 \times 10^6$ SHED were co-cultured with Pan-T cells for 3 days in six-well plates. Flow cytometric analysis (FACSCalibur, BD Bioscience) was utilized to evaluate SHED apoptosis utilizing Annexin V-PE Apoptosis Detection Kit (BD Bioscience). To analyze the role of the elasticity/porosity of the hydrogel biomaterial on SHED-T lymphocyte interaction *in vitro*, $2 \times 10^6$ SHED were encapsulated in 1 mL alginate hydrogel with different degrees of elasticity/porosity. The encapsulated SHED were co-cultured with ($1 \times 10^6$) Pan-T cells for 3 days. After three days of co-culturing, the hydrogels were dissolved in sodium citrate buffer and apoptosis of SHED was evaluated according to the abovementioned method. Six independent specimens (n = 6) were analyzed per group.

### 2.5. Osteo-differentiation of SHED in vitro and the role of inflammatory cytokines

$5 \times 10^5$ SHED were cultured in a six-well plate under the osteogenic condition for 4 weeks while pro-inflammatory