Injectable cell encapsulated hyaluronic acid microgels for nucleus pulposus regeneration

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INTRODUCTION

Low back pain (LBP) is a widespread health problem affecting a considerable proportion of adults worldwide, with estimates suggesting that as many as 80% may experience it[1]. One of the leading causes of LBP is intervertebral disc (IVD) degeneration. Mesenchymal stromal cells (MSCs) offer promising potential to regenerate IVD in the initial stages of degeneration[2]. The conventional method for stem cell delivery is typically done through direct injection into the target tissue or site. Nevertheless, there is an ongoing debate regarding the survival of transplanted cells in cell suspensions within the challenging environment of degenerated IVD, which could negatively impact the effectiveness of cell therapy[3]. Utilizing microgels as carriers for delivering cells shows promise in addressing this challenge. Microgels replicate the three-dimensional natural environment of cells, facilitate efficient substance transfer, and can be administered through injection[4]. Due to its biocompatibility, biodegradability, non-immunogenic, non-toxic, and non-inflammatory properties, hyaluronic acid has been used as a biomaterial for clinical products for over three decades[5]. Herein, injectable microgels based on hyaluronic acid (HA) were developed for MSCs delivery to the IVD.

METHODS

Tyramine-grafted HA (HA-Tyr) was prepared using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and n-hydroxysulfosuccinimide (NHS) chemistry[6]. HA-Tyr solutions with different concentrations (1.5, 2, and 2.5 % w/v) were extruded and microdroplets were generated using a flicking-based vibrating nozzle system. The droplets were directly collected in a H_2O_2 solution and formed microgels. The gelation time and crosslinking conditions were optimized to produce spherical microgels at a high production rate. These cell-encapsulated microgels were utilized for discogenic induction.

RESULTS

With this novel approach, uniform microgels could be fabricated with narrow distribution and spherical shape. Light microscopy images showed that the HA-Tyr microgels have spherical shape morphology within a size range of 505 ± 47µm (Figures 1A). This was also verified by scanning electron microscopy (SEM) as shown in Figure 1B. The resulting HA-Tyr microgels are injectable through needles with conventional gauges (21 G) and disperse rapidly in aqueous media after injection (Figure 1C). Live/dead staining confirmed that the concentration for the crosslinker did not damage the encapsulated cells, as there was abundant green staining, indicating live cells. Also, cell-encapsulated microgels showed an even distribution of cells within the microgels (Figure 1D).

DISCUSSION

This study demonstrates that HA-Tyr-based microgels serve as a reliable system for delivering cells to degenerated IVD by injecting cell-encapsulated microgels. In addition, the current encapsulation procedure can be adjusted to meet the needs of different applications. For instance, it can serve as a dynamic bioreactor system for cultivating MSCs and producing cell-based therapies such as growth factors. Additionally, it can

create *in-vitro* platforms for drug screening and disease modeling or act as bioinks for bottom-up tissue engineering in bioprinting.

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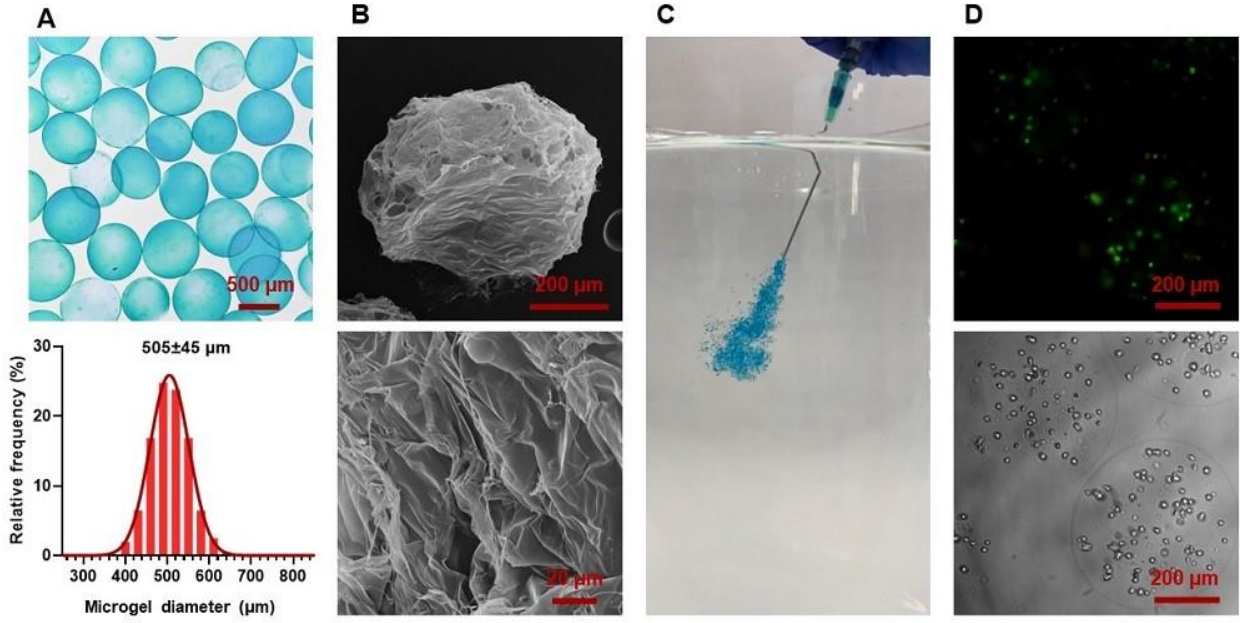

Figure 1. Enzymatically crosslinked HA microgels. (A) light microscopy image and size distribution, (B) SEM images, (C) image of injectability of microgels, and (D) viability of encapsulated MSCs.