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# Influence of viscosity and velocity of administration on the performance of hyaluronic acid as a vehicle for bioprinting and injectable cell therapy: a computer simulation approach and in vitro validation

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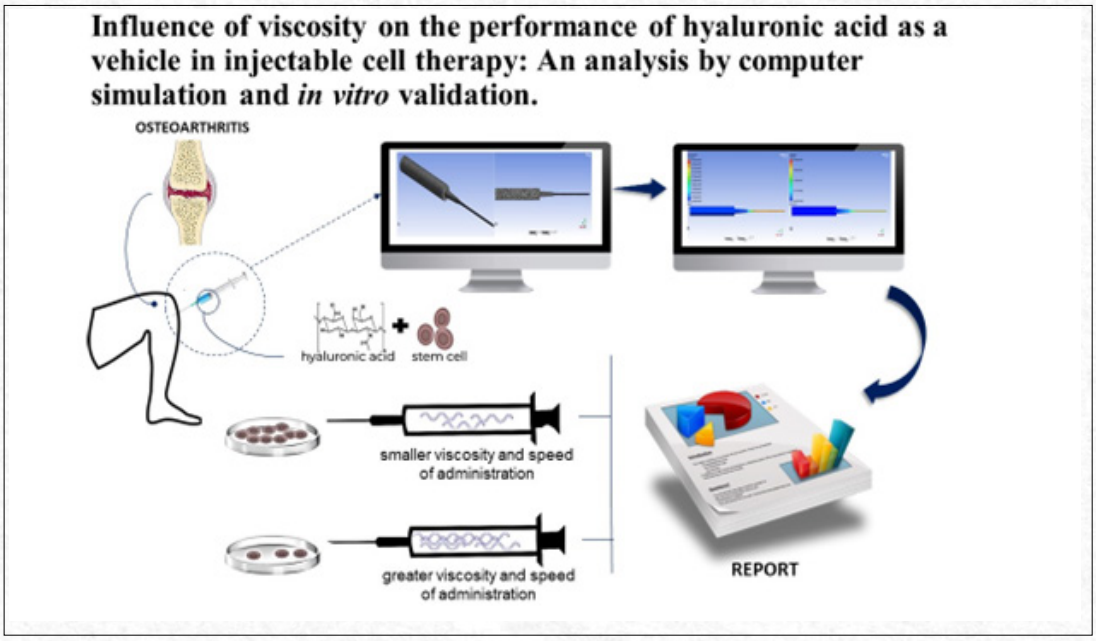


**Abstract:** Background: Hyaluronic acid (HA) is a natural polymer widely used as a vehicle in injectable cell therapy for the treatment of arthropathies. Objective: To estimate, through computational simulations and in vitro validation, the influence of HA's physicochemical properties and administration speed on the shear stress generated in the syringe/needle system, as well as the associated risk to cell viability during administration. Methods: The influence of viscosity was evaluated by considering the rheological parameters corresponding to HA concentrations of 6, 8, 10, 12, and 15 mg/mL. For assessing the impact of administration speed, values representative of the typical speed range used in clinical procedures were considered. Simulations were used to estimate shear stress as a function of administration speed for each viscosity level. Results: The findings revealed a directly proportional relationship between viscosity and administration speed with the magnitude of shear stress. Notably, the highest viscosity formulation, when administered at the fastest speed, reached "critical values" of shear stress associated with mechanical damage to cell membranes and cell death. Conversely, lower viscosity HA exhibited reduced stress levels, indicating it as the potentially preferred formulation for injectable cell therapy. The in vitro cell culture assays corroborated the computational simulation results. Conclusions: The administration of HA demonstrates a viscosity- and speed-dependent effect on shear stress, which should be carefully considered for its application in bioprinting and injectable cell therapies.

**Keywords:** Hyaluronic Acid. Regenerative Medicine. Cell and Tissue Transplant Based Therapy. Computer Simulation. Stem Cells. Bioprinting.

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## Introduction

Hyaluronic acid (HA) is a biopolymer that forms a key component of the extracellular matrix in various tissues of the human body and serves as a fundamental constituent of synovial fluid within synovial joints [1]. Structurally, it is an anionic, non-sulfated glycosaminoglycan that consists of repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid, linked by alternating β-1,4 and β-1,3 glycosidic bonds[2,3]. Due to its high biocompatibility, biodegradability, non-toxicity, and low immunogenicity, HA has gained significant attention over the past decade as a superior biomaterial in diverse fields such as orthopedics, dermatology, aesthetics, tissue engineering, and drug delivery[2,4,5,6,7].

Given its safety profile and proven therapeutic efficacy, particularly in intra-articular viscosupplementation for osteoarthritis, HA has been increasingly used as a preferred carrier in clinical research focused on injectable cell therapy (ICT)[8,9,10]. Specifically, it is employed to deliver mesenchymal stem cells (MSCs) as part of regenerative medicine strategies aimed at treating musculoskeletal pathologies of both traumatic and degenerative origins [3,5,11].

Despite the highly promising preliminary therapeutic outcomes, ongoing ICT research is focused on optimizing cellular parameters and administration procedures to maximize therapeutic benefits [11,12]. Among the critical factors for the success of cell therapy is the availability of a sufficient quantity of healthy and functional MSCs at the repair site. To address this, researchers are actively investigating optimal cell concentrations tailored to specific MSC sources and target pathological conditions. Equally important, though currently less explored, is the need to define parameters related to the carrier vehicle and the procedure itself for ICT administration[13,14].

While HA is widely used as the preferred vehicle for MSC-based ICT, there remains a lack of consistent scientific evidence clarifying its influence on these cells, both during and after the injection procedure. Existing studies, though not specifically focused on ICT applications, indicate that high molecular weight HA may exert modulatory effects on cultured cells. This includes modulation on cell viability, cell proliferation and/or the stimulation of biomolecule release involved in immunomodulation, neovascularization and regeneration within the microenvironment[6,9]. Beyond its potential direct cellular effects, the impact of HA's physicochemical properties on the mechanical environment during ICT administration is still unknown. Such factors could influence cell integrity and potentially compromise therapeutic efficacy. Therefore, this study aims to investigate the influence of HA viscosity, when used as a vehicle in ICT, on cell viability during the administration process. Given the characteristics of the injectable delivery method, the administration speed was also considered a crucial technical variable.

The use of computer simulation has been more and more adopted for analyzing different scenarios and predicting the behavior of some process or some specific variable. Normally, computer simulations are performed after strategical simplification in the model including variables and conditions of interest. Despite it, this tool application is very cheap and avoids waste of time and resources for reaching important conclusions and decisions before a real stuff is indeed implemented [15-18].

## Materials and methods

### Computational Simulation of the Influence of Hyaluronic Acid Viscosity

The initial step involved developing a virtual model to enable computational simulation experiments aimed at determining the influence of varying hyaluronic acid (HA) viscosity and administration speed on shear stress levels affecting the administered cells. The simulation analysis focused on evaluating HA flow behavior within the syringe/needle assembly during administration.

### Obtaining the Geometry and 3D Mesh of Elements

The model was initially designed using computer-aided design software, Rhinoceros® 5.0, based on the geometry of the syringe and needle components of an injection device. This allowed for an accurate reproduction of the syringe body, tip, barrel, and needle shaft. The geometric parameters were provided by DMC Equipamentos LTDA. Specifically, the dimensions used in the analysis corresponded to a 2 mL syringe (Ø = 8.65 mm; length = 64.4 mm) and a 22G 25 x 7 needle (Injex company®). These dimensions are consistent with the administration device accompanying the bioproduct Opus Joint®, which comprises non-animal-origin HA and is used for intra-articular viscosupplementation in osteoarthritis patients.

After establishing the geometry of the syringe and needle components in a virtual environment, a triangular mesh was generated using Ansys/CFX® 18.2 software. The mesh for the inner wall of the syringe/needle assembly contained the smallest elements (Figure 1), enabling more refined analysis in regions with the highest predicted shear stress levels.

The size of the mesh elements was dependent on the geometry's dimensions. Generally, it is recommended to have at least three elements

along the smallest area of interest to ensure a robust simulation. Figure 2 illustrates the variation in size and number of elements in regions where the geometry changes abruptly, particularly around the syringe nozzle, barrel, and needle shaft. The regions corresponding to the barrel and needle shaft featured the smallest elements, enhancing detail in the analysis of these critical areas, where the highest shear stress levels were anticipated during HA flow.

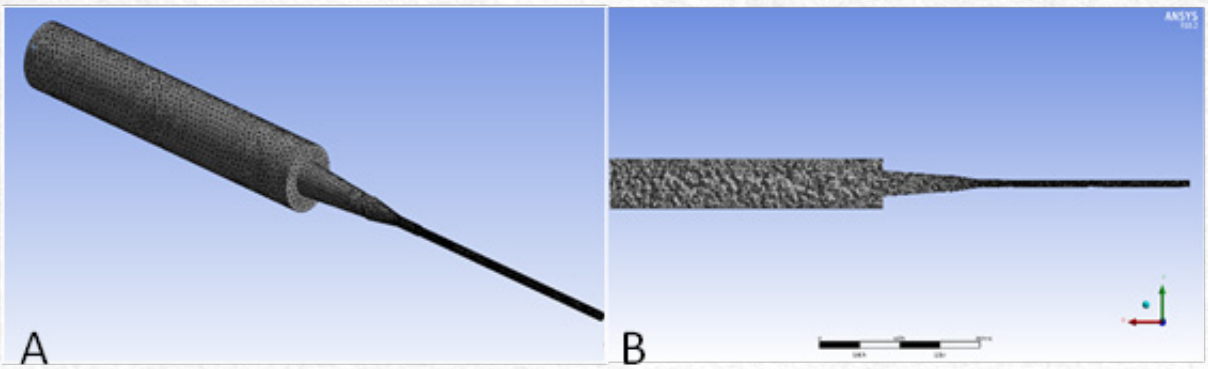


**Figure 1** - Image of the external (A) and internal (B) mesh of the syringe/needle assembly geometry.

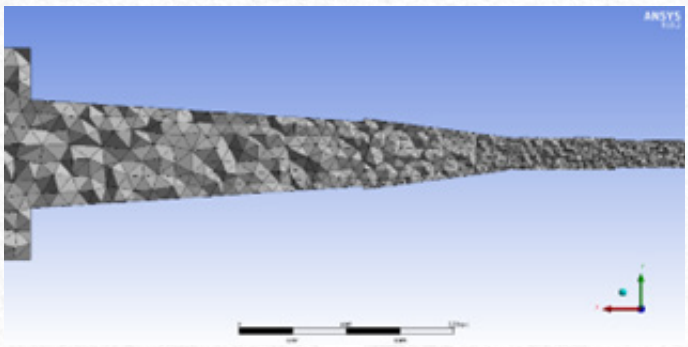

**Figure 2** - Highlighting of the mesh refinement in regions corresponding to the syringe nozzle and needle rod.

**Production and Establishment of Rheological Parameters for Different HA Formulations**

To simulate the influence of HA viscosity, the rheological parameters of various HA formulations were established. One of the formulations analyzed was Opus Joint® itself. Based on its concentration, four additional formulations with varying concentrations were prepared, resulting in a 5-point scale with concentrations of 6, 8, 10, 12, and 15 mg/mL. The rheological parameters for each formulation were provided by DMC Equipamentos. However, specific values and other physicochemical characteristics are not disclosed due to confidentiality agreements with the manufacturer.

HA is classified as a viscoelastic fluid, capable of exhibiting the elastic properties of a solid (approximated using Hooke's Law) as well as the viscosity of a fluid. Depending on the applied stress, it displays both viscous and plastic behaviors simultaneously. Due to its viscoelastic nature, shear stress is reduced by its elastic properties. However, for the purposes of this study, the fluid was treated as a non-Newtonian fluid to simulate critical conditions without attenuating viscous forces.

Unlike Newtonian fluids, the viscosity of non-Newtonian fluids is not proportional to shear stress and changes throughout the flow. This change can be modeled mathematically, and for this study, the power-law model was used, represented by the following equation:

$$\tau = K\left(\frac{du}{dy}\right)^{n-1}$$

Where: τ is the shear stress,  is the fluid consistency index, $\frac{du}{dy}$ is the strain rate and $n$ is the power index.

This model was selected because the viscosity vs. shear stress graphs obtained from rheological analyses were consistent with the behavior predicted by the power-law model, which shows a decrease in viscosity with increasing shear stress. The exact graphs are not presented here to maintain the

confidentiality of the material properties. From these graphs, by normalizing the data using a logarithmic transformation, the values for *K* and *n* were determined.

### Determination of Administration Velocities

A key factor in conducting the simulation was identifying realistic flow velocities. To achieve this, publicly available videos of intra-articular HA viscosupplementation procedures were analyzed. From these videos, application times (Δt) were measured, and with knowledge of the applied volume, the lengths (Δs) corresponding to the syringe geometries were determined. Using these values, the average fluid velocity was estimated for each video using the formula:

$$v = \frac{\Delta s}{\Delta t}$$

Velocity values were derived from five videos (Table 1), and all were tested in the simulations. It was anticipated that higher velocities would result in increased shear stress, allowing for the assessment of the impact of flow speed on shear stress levels.

**Table 1 -** Average velocities obtained from public domain videos during the application of viscosupplementation with HA.

| Velocity | Average velocity (m/s) |
|---|---|
| 1 | $8{,}3\times10^{-4}$ |
| 2 | $1{,}6\times10^{-3}$ |
| 3 | $1{,}9\times10^{-3}$ |
| 4 | $3{,}8\times10^{-3}$ |
| 5 | $6{,}0\times10^{-3}$ |
| 6 | $1{,}3\times10^{-2}$ |

### Determination of shear stress by computer simulation

Simulations were conducted using computational fluid dynamics (CFD) in Ansys/CFX® 18.2, which employs the finite volume method to estimate shear stress in different regions of the syringe/needle assembly. The CFD model for non-Newtonian fluids was based on the Ostwald-de Waele power law. The software required input of the following properties for each HA formulation: material density, molar mass, maximum and minimum shear rates, time constants for viscosity curve generation, fluid consistency index (k=0.2 Pa.s); and the power-law index (n=0.0365).

Once the parameters were configured, the simulations produced plots correlating shear stress with administration speeds for each HA formulation.

### *In vitro validation*

#### *Cell culture*

Human fibroblast cells (GM07492 cell line) were selected for use in this study. The cells were thawed and maintained throughout the experimental phases in flasks containing Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum and antibiotics. The culture environment was strictly controlled, with the incubator set to a temperature of 37°C, in a humidified atmosphere containing 5% $CO_2$.

### Cell viability analysis

In this phase of the project, parameters determined during the computational simulation were validated by comparing in vitro cell viability post-loading, using the optimal HA viscosities and administration velocities identified earlier. To achieve this, cultured human fibroblasts were detached from the culture flasks, resuspended in 2 mL of HA (at a concentration of $10^5$ cells/mL), and loaded into syringe/needle sets identical to those used in the simulation phase. The samples were then administered into wells of a 24-well cell culture plate at rates consistent with those used during the simulations. For the control group, a similar procedure was followed, but cells were resuspended in phosphate-buffered saline instead of HA.

Cell viability post-administration was assessed using direct counting methods with trypan blue staining and an automated cell counter (Bio-Rad®). Statistical analysis of the results was performed using basic descriptive statistics and a one-way ANOVA test, followed by Tukey's post hoc test. A significance level of 5% was used for all analyses.

### Results and Discussion

#### *Determination of Shear Stress by Computer Simulation*

The primary goal of creating the computational model was to estimate the shear stress within the syringe/needle assembly, considering different HA formulations and administration velocities. The expectation was that the simulation would help optimize in vitro biological assays by accurately predicting the influence of key variables (viscosity and administration speed) on the mechanical environment to which the cells would be subjected during the injectable administration procedure.

In addition to optimization, it is important to highlight the potential cost reductions offered by computer simulation, also known as in-silico analysis. This approach reduces the need for extensive in vitro experiments by allowing researchers

to focus on the most relevant experimental conditions for the study's objectives. Such benefits are expected to grow even more in future studies, particularly when human mesenchymal stem cells are used. The advantages of computer simulation in various research fields, including chemistry, biology, biotechnology, and medicine, have been widely recognized in recent decades. Huang and colleagues [19] suggested that CFD could serve as a valuable tool for analyzing and visualizing the impact of fluid forces and stresses on cells. Furthermore, they proposed that computational models will be essential for predicting and testing the large number of parameters that influence cell behavior. The properties of synovial fluid and hyaluronic acid, along with their rheological characteristics, have been well documented in the literature, supporting our proposed model.

Despite the challenges still faced in using computer simulation, such as: the need to validate virtual models, improve reproducibility, advance hardware and software technology, and develop calibration methods, the potential of this approach is widely acknowledged. The expectation is that computer simulations will become increasingly integrated into the scientific methodologies of the aforementioned fields[20]. Regarding the next steps of this research, we aim to gather data that will contribute to the validation of the proposed model, allowing for refinements and improvements to the accuracy and precision of the method.

The shear stress was analyzed at the body and tip walls of the syringe and the cannon and needle shaft. Based solely on the geometry of the syringe/needle assembly, we observed a typical pattern: shear stress was higher in regions with abrupt changes in area and smaller dimensions, as expected. It was clear that the regions of highest shear stress, regardless of HA concentration, were located in the segments corresponding to the cannon and the needle shaft, with the latter exhibiting the most critical shear stress levels.

Figure 3A shows the color plot of shear stress within the syringe/needle assembly during the simulation of HA application with a concentration of 15 mg/mL at a velocity of $1.6 \times 10^{-3}$ m/s. From these results, we identified the needle as the point of greatest risk to cell viability due to the elevated shear stress that could compromise cellular integrity. Figure 3B illustrates the distribution of flow velocity under the same administration conditions, showing an increase in HA flow velocity, in laminar flow, as it moves through the cannon and needle shaft components. Specifically, within the needle shaft, the flow velocity was highest at the center and progressively decreased toward the wall. This behavior was consistent across all HA formulations.

From the compiled simulation data, we were able to establish the relationship between shear stress, HA concentration (viscosity), and administration velocity (Figure 4).

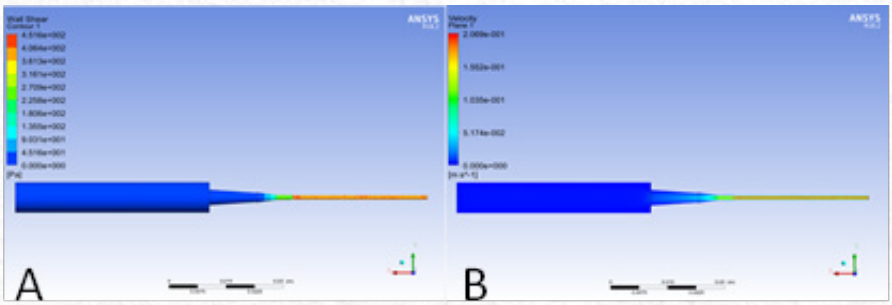


**Figure 3** - Shear stress on the wall of the structure and critical points within the needle with higher cellular risk potentials (A) and distribution of flow velocity on a permanent regime (B).

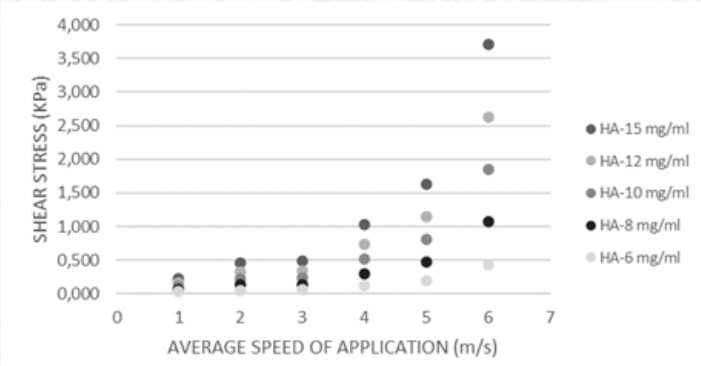


**Figure 4** - Variation of shear stress for the 6 values of velocities and 5 values of HA concentration.

A directly proportional relationship between formulation concentration (and thus viscosity) and shear stress was observed when analyzing each velocity separately. It was also evident that the increase in shear stress was more pronounced with higher administration speeds. These results highlight the concentration/viscosity-dependent and velocity-dependent nature of shear stress generated during the injectable administration of HA.

Another important finding is that the formulation with the highest concentration achieved significantly high shear stress values (3.75 kPa) at the highest administration speed, approaching the "critical value" of approximately 4 kPa proposed by Blaeser et al. [21]. In their study, which assessed the risk of cell death during bioprinting procedures, shear stresses near the "critical value" were shown to be sufficient to induce cell death (decreased cell viability) by damaging the structural integrity of the cytoplasmic membrane. This experiment was conducted using fibroblasts embedded in an alginate hydrogel for extrusion in a bioprinter. Factors such as cell type, hydrogel physicochemical properties, and ejection speed may influence the comparison of results. However, it is important to note that, to our knowledge, no scientific publications have specifically addressed injectable administration of cells for regenerative medicine applications. This is why we have compared our findings to the above reference, despite the technical discrepancies.

### *In vitro validation*

To perform the in vitro experiments, the lowest (8.3 x $10^{-4}$ m/s) and highest (1.3 x $10^{-2}$ m/s) velocity values were selected, corresponding to HA concentrations of 6 mg/mL and 15 mg/mL, respectively. These values were derived from the computational simulation results. As mentioned earlier, the 6 mg/mL concentration resulted in low shear stress values, regardless of the administration speed. The 15 mg/mL concentration was included in the analysis to assess the real risk of cell death under conditions that produced the highest stress levels. Injectable administration simulations were conducted manually to achieve a higher clinical correlation (hypothetically) for cell therapy administration. The administration time was used as the reference parameter to proportionally infer the administration speed predicted in the simulation.

Data obtained from in vitro experiments enabled the assessment of cell viability, expressed as a percentage, for each experimental group following the simulated administration procedure. These results were subsequently compared to one another and to the control condition (Figure 5).

The control group exhibited cell viability with a mean and standard deviation (M±SD) of 96.3±4%. These high values are consistent with administration via a liquid vehicle (PBS), which induces minimal shear stress. Values slightly below 100% were expected, reflecting normal cell viability loss associated with standard culture handling procedures. For the HA groups at a concentration of 6 mg/mL administered at low and high speeds, viability was 82.7±4% and 88.7±5%, respectively. While these means were marginally lower than those of the control group, the differences were not statistically significant ($p \geq 0.05$). In contrast, the HA groups at 15 mg/mL showed viability of 54.7±8% and 36±4.6% for the low and high-speed administrations, respectively. Both values were significantly lower ($p < 0.0006$) than those for the control and the HA 6 mg/mL groups, independent of speed. Additionally, a statistically significant difference ($p \leq 0.01$) was observed between the HA 15 mg/mL groups administered at different speeds, with higher speeds resulting in lower viability.

Blaeser et al. [21] investigated cell viability as a

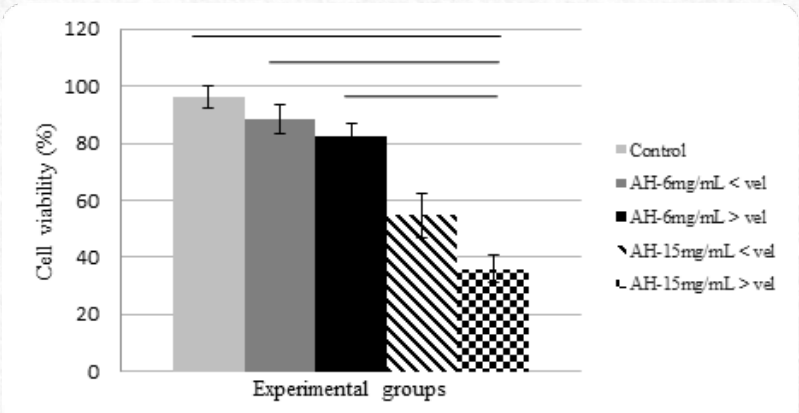


**Figure 5** - Mean and standard deviation values of cell viability (human fibroblasts - GM07492) found after injectable administration for the Control, 6 and 15 mg/mL HA groups analyzed (highest and lowest speed) * $p < 0.0006$.

function of shear stress within a 3D printing nozzle, categorizing shear into three groups: <5 kPa, 5-10 kPa, and >10 kPa. They found that cell viability for the <5 kPa group was 96%, indicating preservation of membrane integrity under low shear stress. However, viability significantly declined for groups exposed to >5 kPa shear (91% and 76%, respectively). Notably, their study did not assess the effect of application speed, and since bioprinting typically involves lower velocities than manual administration, it can be inferred that their data align with our findings.

The absence of statistically significant differences in viability among the control, HA-6mg/mL<vel, and HA-6mg/mL>vel groups suggests that cells exhibit a high tolerance to shear stress levels generated during injectable administration, preserving structural integrity. These findings are consistent with low shear stress levels predicted by computational simulations.

In contrast, the significant reduction in viability observed in the HA-15mg/mL<vel and HA-15mg/mL>vel groups suggests that shear stress levels reached thresholds critical to cell integrity during administration. The notably lower viability in the HA-15mg/mL>vel group aligns with high shear stress levels predicted in simulations. Here, cell death reached approximately 65%, which could severely compromise the therapeutic efficacy of the technique, as it relies on maintaining high numbers of viable cells post-administration. Previous studies by Blaeser et al.[21] and Chang et al.[22] also reported reduced viability, although with lower percentages of cell death under lower velocity conditions typical of bioprinting.

The significant viability reduction in the HA-15mg/mL<vel group, despite low predicted shear stress levels, suggests that actual shear stress during in vitro experimentation may have been higher than expected. Although this discrepancy was considered during experimental planning, it was deemed a lower priority to more closely mimic real-world injectable administration.

The in vitro viability results of this study support the use of HA at 6 mg/mL concentration as optimal for ensuring high cell viability following injectable administration. However, additional research is needed to explore not only the risk of cell death due to high shear stress but also the potential impact of even low shear levels associated with HA concentration on cell viability.

Kim et al.[23] demonstrated that shear stress induced by interstitial flow promotes osteogenic differentiation of mesenchymal stem cells via TAZ receptor activation, with calculated shear stress around 0.0135 Pa. This low-level shear stress, achieved experimentally using microchannel flow systems, could enhance cell differentiation and was reached by all HA formulations even at reduced administration speeds.

The theoretical basis of mechanobiology, initially proposed by Friedrich Pauwels, links mechanical stimuli to cell differentiation processes. This concept strengthens the hypothesis that the mechanical environment during injectable administration may have beneficial effects beyond mere cell viability, potentially enhancing MSC-based therapies [4,24]. Further research is warranted to explore the balance between beneficial mechanobiological stimuli and the risk of cell damage, as well as the interaction between HA macromolecules and cells during administration.

## Conclusions

The findings of this research provided a detailed characterization of the relationship between shear stress—linked to the viscosity and flow velocity of HA—and cell viability post-administration. The low levels of shear stress predicted by computational simulations, combined with the high cell viability observed in in vitro assays, suggest that a viscosity corresponding to an HA concentration of 6 mg/mL is the most promising for use as a bioink (HA+cells) in bioprinting and for injectable cell therapy applications. Additionally, these results highlight the significant potential of computational simulations as tools for planning and optimizing experimental research in biological sciences, health sciences, and biotechnology. However, further studies are required to fully elucidate the impact of the mechanical environment generated during injectable administration on cellular structural integrity and viability. These efforts are crucial to enhancing the safety and therapeutic efficacy of injectable cell therapies.

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