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Study on the Protective Effect of Low-Stress Damping Pipette Connector for Fragile Oral Samples

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Abstract:

Oral samples consist of primary gingival cells, saliva, and dental plaque microbial communities, featuring complex compositions. Their cells and microorganisms are highly sensitive to fluid shear force. When samples are directly aspirated and dispensed by conventional pipettes, high velocity liquid flow generates considerable fluid shear stress, which leads to rupture of gingival cells and mechanical damage to microorganisms, thereby confounding subsequent experimental outcomes such as cell culture and 16S rRNA sequencing. Commercially available slow aspiration pipette connectors achieve flow rate damping via tiny central capillary orifices. However, such orifices are prone to clogging when handling high viscosity samples rich in mucin like saliva, limiting their applicability to pre processing of oral samples. In this paper, a low stress damping pipette connector with labyrinth type flow channels is designed. Equipped with widened circuitous labyrinth channels and bottom shunt guide teeth, the device buffers aspiration dispensing flow velocity while avoiding clogging risks posed by high viscosity saliva samples. Its protective performance for fragile oral samples is evaluated through fluid impulse calculation, cell damage control experiments, and anti clogging simulation tests for high viscosity samples. The results demonstrate that the proposed device markedly mitigates fluid impact during pipetting and alleviates mechanical damage to primary gingival cells. It reduces mechanical lysis of simulated oral microbes and improves microbial DNA recovery, which is beneficial for downstream experiments such as 16S rRNA sequencing. The entire device is autoclavable, compatible with existing commercial pipettes, and requires no instrument modification for operation. It provides a low cost hardware solution for standardized pre processing of oral samples.

Keywords: Fluid shear stress, Oral samples, Primary gingival cells, Dental plaque, Sample pre processing.

1. Introduction

Oral microbiome and primary cells from gingival tissues are essential experimental materials for stomatological research, widely adopted in studies on the pathogenesis of periodontal diseases, oral microecological imbalance, and mechanisms underlying oral tumors[1]. Within laboratory sample pre-processing workflows, pipettes represent the most fundamental and frequently-used operating tools. Nevertheless, fluid shear stress generated during aspiration and dispensing can induce non-negligible mechanical damage to fragile cells and microorganisms[4]. When standard pipette tips aspirate and dispense liquids, concentrated high-velocity flow leads to lysis of primary cells. Some symbiotic microorganisms with low tolerance undergo lysis and death under fluid impact, distorting community abundance in sequencing outputs and introducing considerable systematic errors into downstream experiments[5]. Existing studies have indicated that operational biases introduced

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at the sample pre-processing stage constitute one major cause of poor reproducibility of oral microbiome results across different laboratories[6].

To mitigate pipetting-induced mechanical damage, available solutions include wide-orifice tips, variable--speed electronic pipettes, and threaded capillary slow-aspiration connectors[7].Wide-orifice tips are prone to liquid leakage when handling saliva samples with low surface tension. Electronic pipettes incur high procurement costs, and certain models cannot withstand autoclaving, making them unsuitable for sterile cell manipulation. Capillary-based slow-aspiration connectors reported in existing patents rely on tiny central orifices for flow damping. When processing mucin-rich samples such as saliva and dental plaque, mucin deposits readily block the micropores, preventing continuous processing of oral specimens[8]. Saliva contains abundant mucins and glycoproteins, and sample viscosity varies greatly among individuals. This accounts for why well-established consumables from general biology laboratories often fail when directly applied to oral sample processing[9]. Domestic guidelines for oral sample pre-processing only give general recommendations for “gentle pipetting”. Quantified standards for flow velocity and shear-stress control are absent. Operations rely heavily on personal experience of experimenters, posing difficulties for novice operators and resulting in large intra-laboratory and inter-laboratory operational variations[10]. At present, specialized pipetting-buffering consumables tailored for high-viscosity fragile oral samples are scarce in China. Targeting the limitations of existing technologies, this study designs a low-stress damping pipette connector with labyrinth--type flow channels. Abandoning conventional central capillary damping structures, the device dissipates fluid kinetic energy through widened circuitous microfluidic labyrinth chambers. Circumferentially distributed guide teeth at the bottom disperse liquid flow so as to reduce concentrated impact upon liquid ejection. An axial positioning step inside the connector fits short--type tips commonly used in oral laboratories. Fabricated from polypropylene, the whole device tolerates autoclaving at 121℃. Fluid-performance evaluation and mock-sample validation are carried out in this work to explore its application value in pre-processing fragile oral samples, and to provide hardware references for standardized manipulation of oral specimens.

2. The Association Between MODY and the Immune System

2.1. Device Structure and Fluid Simulation

The low-stress damping pipette connector is installed between the pipette port and the pipette tip. Its main components include the pipette connection end, tip-socket end, microfluidic damping labyrinth chamber, turbulence damping plate, multi-stage flow-guiding buffer outlet, dispersed flow-guiding teeth, annular positioning step and rotary adjustment structure. The whole connector is integrally injection-molded from polypropylene (PP), and can withstand autoclaving at 121℃. The labyrinth chamber adopts widened flow channels with a minimum flow cross-section greater than 1.2 mm² to avoid channel clogging caused by salivary mucins. The rotary adjustment structure changes the effective flow area of the turbulence damping plate to continuously adjust the damping intensity during aspiration and dispensing. The rotary adjustment structure can alter the effective flow area of the turbulence damping plate to achieve multiple damping adjustment settings, which correspond to different aspiration dispensing flow rates.The bottom dispersed flow-guiding teeth split a single liquid stream into multiple dispersed streams to reduce shear stress induced by dispensing impact.

2.2. Main Reagents and Instruments

100-1000 μL commercial manual pipettes (Dragon Lab, Eppendorf); standard pipette tips; human gingival primary cells (HGC, purchased from iCell Bioscience); simulated saliva (containing 2.5 g/L porcine submandibular mucin, prepared according to reported methods[12]); PBS buffer; complete culture medium; 0.25% trypsin EDTA digestion solution; cell counter; inverted optical microscope; high speed camera (240 frames/s); SPSS 26.0 statistical software.

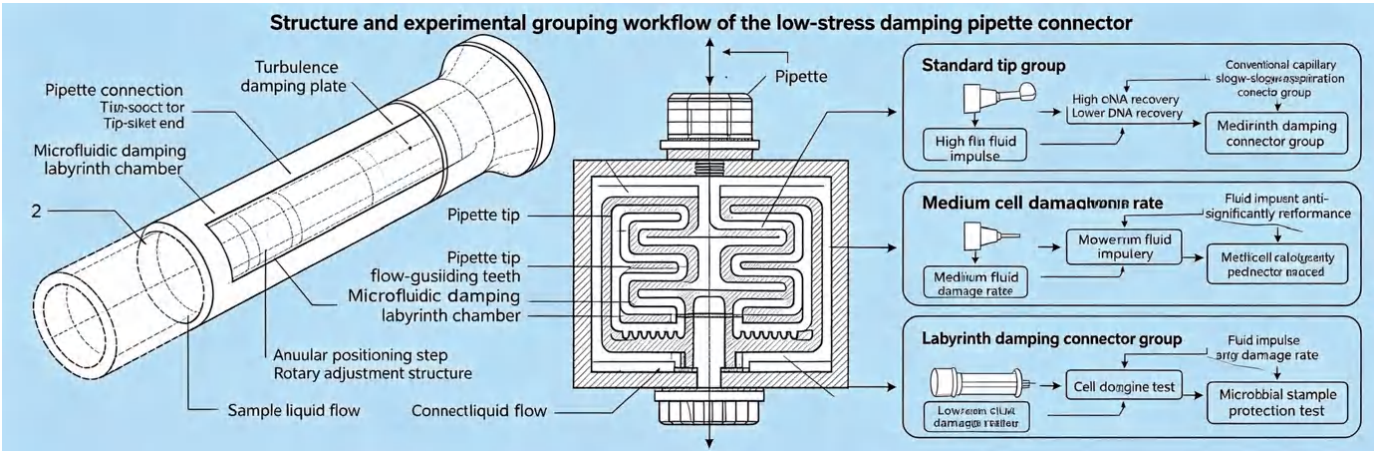


Figure 1. Structure of low stress damping pipette connector and experimental grouping workflow. (Graphical Abstract)

2.3. Fluid Impulse and Flow Velocity Test

Standard tips, conventional capillary slow aspiration connectors, and tips assembled with the proposed damping connector were used to aspirate 300 μL PBS. The whole dispensing process was recorded by high speed camera. Post processing of video footage was applied to calculate the average outlet flow velocity and fluid impulse, so as to compare fluid impact intensity among the three groups. Six technical replicates were performed for each group[13]. Meanwhile, the adjustment gear of the device was rotated to record the average aspiration and dispensing flow velocity under different damping gears, verifying its continuous flow rate adjustment function.

2.4. Control Experiment on Human Gingival Primary Cell Damage

Well conditioned human gingival primary cells were digested and resuspended, and the cell suspension concentration was adjusted to 100,000 cells/mL. Samples were divided into three groups: Group A (control group, standard pipette tip), Group B (conventional slow aspiration connector group), Group C (experimental group with the labyrinth damping connector). Unified pipetting procedure: aspirate 300 μL cell suspension, and repeat aspiration dispensing for 5 times to simulate laboratory resuspension operations[14]. After treatment, total cell number, viable cells and broken cells were counted under a microscope to calculate the cell damage rate. Three biological replicates were set for each group, with three technical replicates per biological replicate.

Cell damage rate = (number of broken cells / total cell number) × 100%.

2.5. Anti Clogging Performance Test for High Viscosity Samples

Mucin containing simulated saliva samples were prepared. Conventional capillary slow aspiration connectors and the labyrinth channel connector were used for 20 consecutive cycles of aspiration dispensing with a single volume of 400 μL. After each cycle, internal channels were inspected by naked eyes combined with microscopic observation. The occurrence times and operation rounds of clogging or obvious flow decline were recorded to compare the anti clogging capacity of the two groups[15].

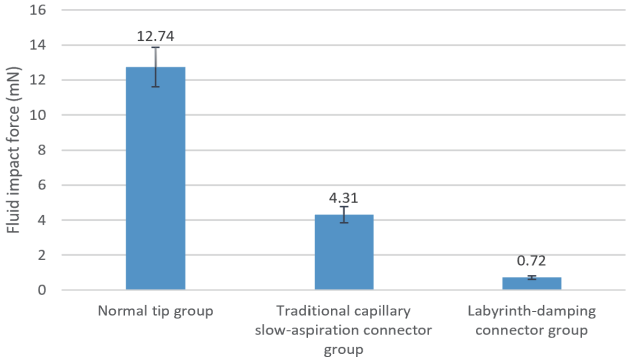


Figure 2. Comparison of outlet fluid impact force under three pipetting conditions

2.6. Preliminary Experiment on Protective Effect for Simulated Oral Microbial Samples

A mixed suspension of simulated oral microorganisms (mixture of *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and standard *Streptococcus strains*) was prepared. Gentle pipetting resuspension was performed 5 times using standard tips and the proposed damping connector respectively. Total microbial DNA was extracted after operation for DNA integrity detection. qPCR was adopted to quantify the copy number of target strains. Total microbial DNA recovery rate and relative abundance variation of target bacteria after treatment were compared between groups, to preliminarily evaluate the disturbance of pipetting shear impact on microbial communities.

2.7. Statistical Analysis

Statistical analyses were carried out using SPSS 26.0 software. For each biological replicate, values from technical replicates were averaged prior to statistical analysis.Measurement data were expressed as x±s. One way analysis of variance (ANOVA) was used for multi group comparison, and LSD t test was adopted for pairwise comparisons between groups. $P<0.05$ was considered statistically significant.

3. Results

3.1. Results of Fluid Simulation and Fluid Impulse Test

Fluid simulation results revealed high flow velocity and large wall shear stress at the outlet of standard pipette tips. For the conventional capillary connector, mucin retention and accumulation occurred around micropores under simulated saliva conditions, indicating potential clogging risks. Within the widened labyrinth channel of the proposed device, fluid velocity was dissipated step by step, and outlet liquid flow was split by flow guiding teeth, leading to a remarkable reduction in instantaneous outlet impulse.

Measured fluid impulse values were as follows: standard tip: (12.74±1.12) mN; conventional capillary slow aspiration connector: (4.31±0.47) mN; the present labyrinth damping connector: (0.72±0.11) mN. Compared with the standard tip, this connector reduced fluid impact by more than 94%, with statistically significant difference ($P<0.001$). The rotary adjustment structure enabled continuous damping modulation, yielding adjustable aspiration dispensing flow velocity ranging from 0.20 0.52 mL/s, so that appropriate flow rate gears could be matched for different oral samples.

3.2. Comparison of Damage Rates of Primary Gingival Cells

After pipetting treatment, the cell damage rates of primary gingival cells in the three groups were: Group A (standard tip control group): 24.7±2.1%; Group B (conventional capillary slow aspiration connector group): 11.4±1.6%; Group C (experimental group

using the labyrinth damping connector): 5.3±1.2%. One way ANOVA showed an overall statistically significant difference among the three groups (F=127.36, P<0.001). Pair wise comparisons demonstrated that the cell damage rate of Group C was significantly lower than those of Group A (P = 0.0023) and Group B (P = 0.0041). These results indicated that the device could markedly alleviate pipetting induced mechanical cell damage and improve the survival rate of fragile primary cells.

3.3. Anti Clogging Performance for High Viscosity Samples

For the conventional central capillary damping connector, micropore clogging and obvious flow attenuation emerged after 6 9 cyclic aspiration dispensing cycles of simulated saliva. Mucin accumulation at tiny orifices was consistently observed across repeated experiments. By contrast, after 20 consecutive aspiration dispensing cycles of high viscosity simulated saliva using the labyrinth channel connector, no obvious mucin deposition or channel clogging was observed, and the inflow outflow rate remained stable. It was verified that the widened labyrinth channel structure could effectively resist clogging triggered by salivary mucins, and was suitable for continuous processing of high viscosity oral samples such as saliva.

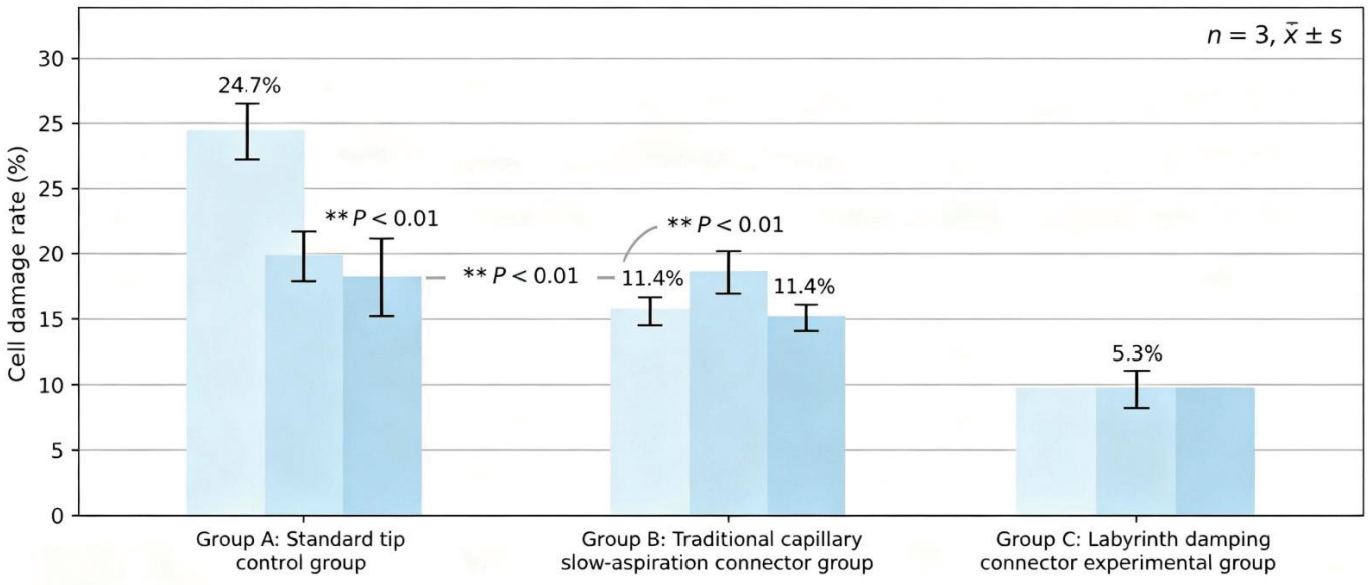
3.4. DNA Recovery Rate of Simulated Microbial Samples

After repeated pipetting, the total microbial DNA recovery rate of the standard tip group was (62.3±4.2)%, whereas that of the damping connector experimental group reached (87.5±3.7) %, with statistically significant difference between the two groups (P=0.0276). qPCR results suggested that the copy numbers of some shear sensitive anaerobic bacteria decreased sharply after manipulation with standard tips, implying mechanical shock mediated microbial lysis and death. When the low stress damping connector was applied, the decline in copy numbers of target microbial populations was substantially mitigated, confirming its protective effect on simulated oral microbial communities.

4. Discussion

Oral derived samples confront two practical challenges during laboratory pre processing. On one hand, primary gingival cells and certain oral commensal anaerobic bacteria are susceptible to fluid shear stress. High velocity liquid flow generated by pipetting may trigger cell lysis and death, which directly compromises outcomes of cell culture and microbiome sequencing. On the other hand, abundant mucins and protein precipitates in saliva readily clog tiny channels. Many slow aspiration consumables that perform well in general cell experiments cannot be directly transferred to the pre processing of oral samples. Most domestic and international literature and operation manuals concerning oral sample handling merely recommend “gentle pipetting”, without quantified criteria for flow velocity and shear stress. Manipulation quality heavily relies on operators’ personal experience, leading to substantial intra and inter laboratory operational variations. This constitutes one critical contributor to poor reproducibility of oral related experimental results[2].

Instead of adopting the conventional central capillary micropore damping strategy, the present device adopts a combined design of widened circuitous

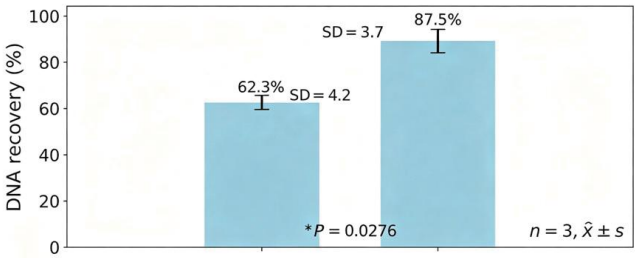


Experimentall data	Cell damage rate (%)	Standard deviation
Group A:	Standard tip control group	24.7 2.1
Group B:	Traditional capillary slow-aspiration connector group	11.4 1.6
Group C:	Labyrinth damping connector experimental group	5.3 1.2

Figure 3. Comparison of cell damage rate of Porphyromonas gingivalis after pipetting treatment. n=3 biological replicates, with three technical replicates per biological replicate; data are presented as x±s.

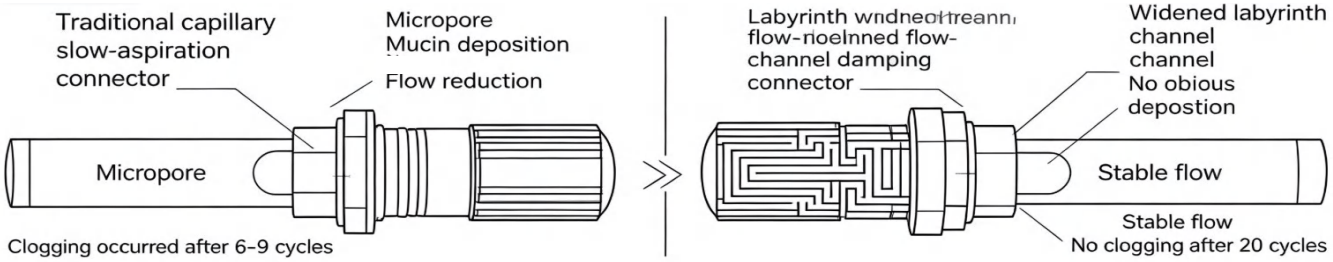
labyrinth channels and bottom flow splitting teeth. Multiple turbulence damping plates inside the labyrinth dissipate fluid kinetic energy and reduce overall aspiration dispensing flow velocity. The bottom flow guiding teeth split concentrated liquid jets into multiple soft dispersed streams, further eliminating localized impact shear stress at the moment of liquid ejection. Dual mechanisms jointly mitigate mechanical damage to samples. The widened channels fundamentally reduce the risk of clogging induced by mucin deposition, overcoming the drawbacks of traditional slow aspiration connectors when handling saliva samples. Furthermore, the whole device made of polypropylene (PP) can be autoclaved at 121℃, compatible with aseptic manipulation of pathogenic microbial samples. An annular positioning step is added at the tip socket end to fit short size tips commonly used in oral laboratories. As an external accessory, it requires no modification to existing pipettes and can be used after direct assembly. Its low unit production cost facilitates large scale popularization in university based oral laboratories and clinical laboratories of dental hospitals, offering a feasible solution for hardware based standardization of pipetting operations for oral samples[3].

volume ranges (10 μL, 200 μL). Further experiments using clinical samples combined with 16S rRNA high throughput sequencing are required to comprehensively evaluate its protective performance against real world clinical oral microbial communities. Future work may establish recommended reference thresholds of flow velocity shear stress for different oral samples, so as to formulate a complete standardized operating procedure for oral sample pipetting.



Group	DNA recovery (%)	Standard	deviation
Standard tip control group		62.3	4.2
Labyrinth damping connector experimental group		87.5	3.7

Figure 5. Comparison of DNA recovery of simulated oral microbiota samples under different pipetting treatments



Anti-clogging performance comparison

Group	Cycle number when clogging occurred	Clogging phenomenon	Clogging phenomenon
Traditional capillary slow-aspiration connector	6-9	Mucin accumulation in micropores, obvious flow reduction	>20
No clogging, stable flow rate	6-9	Labyrinth widened flow-channel damping connector	No clogging, stable flow rate

Figure 4. Comparison of anti clogging performance between traditional capillary slow aspiration connector and labyrinth damping pipette connector

Compared with electronic pipettes, this damping connector requires neither charging nor routine calibration, and its procurement cost is far lower than that of variable speed electronic pipettes. In contrast to standard wide orifice tips, the proposed device actively dissipates fluid kinetic energy to achieve controllable low flow rates, whereas wide orifice tips can only partially reduce local shear without actively limiting liquid flow velocity. Nevertheless, the device has certain limitations. Validations in this study are mainly performed using in vitro simulated saliva, cells and mixed suspensions of reference strains; large scale sequencing verification with clinically derived authentic saliva and dental plaque samples has not yet been carried out. Connectors of matching specifications need to be developed for pipettes of different

5. Conclusion

The low stress damping pipette connector with labyrinth type flow channels designed in this study can effectively reduce fluid shear stress generated during pipetting and mitigate mechanical damage to primary gingival cells. Its widened labyrinth channels resist clogging induced by salivary mucins, making the device suitable for the pre processing of high viscosity oral samples. Compatible with mainstream commercial manual pipettes and autoclavable, it provides a low cost and standardized hardware solution for sample pre processing in oral cell and oral microbiome experiments. It helps reduce experimental variations caused by human operation and improves the reproducibility of oral related experiments.

6. References

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