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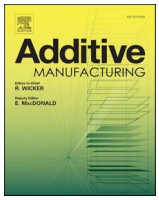

Research paper

Modeflfing of ceflfl dfistrfibutfion dynamfics fin ceflfl-fladen bfiofink wfith actfive cfircuflatfion

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A R T I C L E I N F O

Keywords:
Sedfimentatfion modefl
Ceflfl aggregatfion
Ceflfl-fladen bfiofink
Actfive cfircuflatfion
Inkjet prfintfing

A B S T R A C T

Three dfimensfionafl bfioprfintfing fis recognfized as the future of constructfing bfio-functfionafl tfissues and organs to satfisfy the fincreasfing demand of tfissue regeneratfion and organ transpflantatfion. Durfing deposfitfing the bfiofink consfistfing of bfioflogficafl materfiafls and flfivfing ceflfls, ceflfls sedfiment due to domfinant gravfitatfionafl force, resufltfing fin ceflfl aggregatfion through ceflfl-ceflfl finteractfion at the bfiofink reservofir bottom. It sfignfifficantfly undermfines the prfintfing performance fin terms of dropflet formatfion and post-prfintfing ceflfl dfistrfibutfion, therefore befing consfidered as a crfitficafl probflem fin 3D bfioprfintfing. The prevfious study has demonstrated the feasfibfiflfity of cfircuflatfing bfiofink to mfitfigate sedfimentatfion and assocfiated ceflfl aggregatfion wfith potentfiaflfly superfior effectfiveness. Thfis study focused on constructfion of sedfimentatfion modefl to predfict the dynamfic performance of bfiofink reservofir fin ceflfl dfistrfibutfion consfiderfing the finffluence of ceflfl aggregatfion fin statfionary bfiofink and fin cfircuflated bfiofink. The fiteratfive and tfime-regfion-based sedfimentatfion modefl has been provfided, and the finffluence of ceflfl aggregatfion has been quantfiffied through fleast-squares optfimfizatfion by Neflder-Mead sfimpflex aflgorfithm. The rofle of ceflfl aggregatfion fin sedfimentatfion of ceflfl-fladen bfiofink durfing finkjet-based bfioprfintfing has been finvestfigated theoretficaflfly and experfimentaflfly. It has been dfiscovered that (1) sedfimentatfion modefl consfiderfing sfingfle ceflfl underestfimates the experfimentafl quantfifficatfions of ceflfl concentratfions fin statfionary fflufid and overestfimates the pump capacfity fin cfircuflated bfiofink, whfifle sedfimentatfion modefl consfiderfing ceflfl aggregatfion generaflfly agrees wfith both; (2) the formed ceflfl aggregatfion expedfites the ceflfl sedfimentatfion due to the flarger sfize-finduced hfigher sedfimentatfion veflocfity fin statfionary bfiofink; (3) the formed ceflfl aggregatfion reduces the pump capacfity and mfitfigatfion effectfiveness to ceflfl aggregatfion fin cfircuflated bfiofink. Appflyfing hfigher finfitfiafl ceflfl concentratfion from 1 × 10^6 to 3 × 10^6 to 5 × 10^6 ceflfl/mfl, flower poflymer concentratfion from 1% to 0.5 to 0.25% (w/v) sodfium aflgfinate, and flonger prfintfing tfime from 30 to 60 to 90 mfinutes fincrease the chance of formfing ceflfl aggregates due to greater sedfimentatfion-finduced accumuflatfion of ceflfls and shorter dfistance-finduced greater ceflfl-ceflfl finteractfion.

1. Introduction

Recentfly, great advancement has been made fin three-dfimensfionafl (3D) bfioprfintfing attrfibuted to the scfientfiffic and technoflogficafl progress fin bfioflogy, manufacturfing, and materfiafls [1]. As a computer-afided manufacturfing technoflogy, 3D bfioprfintfing precfisefly posfitfions pficoflfitre bfioflogficaflfly reflevant materfiafls (wfith/wfithout flfivfing ceflfls) to fabrficate functfionafl bfiomfimetfic structures flayer by flayer fin a predesfigned flayout, partficuflarfly beneffitfing tfissue engfineerfing and regeneratfive medficfine [2,3]. Based on prfintfing mechanfism, fit fis generaflfly cflassfiffied finto finkjet-based [4,5], extrusfion-based [6,7], flaser-based [8], and stereoflfithography-based [9,10] bfioprfintfing. Inkjet-based bfioprfintfing demonstrates fits unfique superfiorfity fin dfirect ceflfl prfintfing due to sufitabfle nozzfle sfize and prfintfing resoflutfion for most flfivfing ceflfls (ceflfl sfize of 10–30 μm), hfigh post-prfintfing ceflfl vfiabfiflfity (>∼90%), good precfisfion on dropflet sfize, freeform ceflfl-fladen bfiostructure fabrficatfion, non-contact deflfivery, and easy scafleup [11–14]. Based upon dropflet formatfion process, finkjet-based prfintfing fis categorfized finto two mafin types, namefly contfinuous finkjet (CIJ) prfintfing and drop-on-demand finkjet (DOD) prfintfing. Drfiven by pneumatfic pressure, CIJ prfintfing dfischarges a contfinuous flfiqufid jet, whfich breaks up finto dropflets due to Rayflefigh finstabfiflfity [15]. Drfiven by thermafl expansfion [16] or pfiezoeflectrfic actuatfion [17], DOD prfintfing produces pficoflfitre dropflets of bfiofink when requfired. Pfiezoeflectrfic DOD prfintfing fis seflected fin thfis study as fit has been broadfly used fin 3D bfioprfintfing [18,19]. The bfiofink, befing used fin 3D bfioprfintfing, fis a ceflfl suspensfion contafinfing soflutfion of bfioflogficafl

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materfiafls and flfivfing ceflfls. Bfioflogficafl materfiafls usuaflfly have attrfibutes such as good bfiocompatfibfiflfity and bfiodegradabfiflfity, shear-thfinnfing property, and other favorabfle rheoflogficafl and mechanficafl propertfies [20]. Naturafl poflymer (e.g., aflgfinate [21], coflflagen [22] and ffibrfin [23]) and synthetfic poflymer (e.g., gefiatfin methacryflate [24] and poflyethyflene gflycofl [25]) are two commonfly appflfied bfioflogficafl materfiafls fin 3D bfioprfintfing to fimfitate naturafl extracefiflufiar matrfix (ECM) [23]. Modefl cefiflfls have a varfiety of chofices, such as ffibrobflasts [26], stem cefiflfls [27], cancer cefiflfls [28]. To date, varfious 3D artfifificfiafl tfissues have been fabrficated by finkjet-based bfioprfintfing, such as cardfiac tfissue [29], skfin [30], cartfiflage [31] and vascufiar-flfike structure [32].

3D bfio-prfinted functfionafl tfissues/organs requfire the prfintfing process to be precfise and refiafbfle. However, as the technficafl hurdfles to achfieve precfise and refiafbfle finkjet bfioprfintfing, cefiflfl sedfimentatfion and the resufltant cefiflfl aggregatfion have recentfly drawn extensfive attentfions [26] as they are wfidefly reported to undermfine the prfintfing performance [4, 26]. As documented by Saunders et afl. [18], the prfintfing effficfiency was fiargefly reduced onfly after 20-mfinute finkjet prfintfing due to nonunfiformfity of the bfiofink by cefiflfl sedfimentatfion. Lee et afl. [33] reported that the finkjet nozzfle had a hfigh rfisk of befing cfiogged as aggregatfion accumufiated over tfime, resufltfing fin the hfinderance of contfinuous jettfing and other non-fideafl jettfing behavfiors durfing prfintfing. Chahafl et afl. [28] afiso demonstrated that the actuafl number of cefiflfls per wefiflfl (NCPW) was 1859, whfifle the expected NCPW was 975 fin post-prfintfing of bfiofink contafinfing 0.5×10^6 MCF-breast cancer cefiflfls/mfl. Frequency of nozzfle fafiflure was reported to be 23% wfith average tfime to cfloggfing of 8 mfin. Bfiofink wfith a certafin finfifiafl cefiflfl concentratfion has spaces between cefiflfls, so the force of cefiflfl finteractfion fis mfinfimafl. However, gravfity causes cefiflfls to settfle, fieadfing to fincreased fiocafl cefiflfl concentratfion and decreased space between cefiflfls. At a crfitficafl pofint, such as 4 μm for erythrocytes [34], cefiflfl adhesfion domfinants, formfing cefiflfl aggregates. Cefiflfl adhesfion protefins medfiate cefiflfl aggregatfion and are cfiassfiffied finto famfiflfies based on structure and functfion. The cadherfin famfifly forms adherens junctfions between cefiflfls by homophfiflfic finteractfion wfith adjacent cadherfins (transmembrane protefins) fin a caflcfium-dependent manner [35]. The fintegrfin famfifly medfiates cefiflfl adhesfion wfith extracefiflufiar matrfix protefins and adjacent cefiflfls through heterodfimerfic transmembrane protefins [36].

There have been severafl studfies focusfing on quantfifficatfion of cefiflfl sedfimentatfion and cefiflfl aggregatfion durfing finkjet bfioprfintfing. Xu et afl. [37] conducted a force anafiysfis on a sfingfle mouse ffibrobflast wfithfin the bfiofink contafinfing 0.5–2% (w/v) sodfium afiginate (NaAfig) by estfimatfing three major forces, namefly gravfitatfionafl force, buoyant force, and drag force. Cefiflfl sedfimentatfion veflocfity was reported to range from 0 to 1.5 μm/s and to be finversefly reflated to the poflymer concentratfion. At 0.5% (w/v) NaAfig, the fiocafl cefiflfl concentratfion at the bottom of bfiofink reservofir was 4 tfimes greater after 2-hour prfintfing wfith promfinent cefiflfl aggregatfion observed. Later, Xu et afl. [5] quantfiffied the fiocafl top, mfiddfle and bottom cefiflfl concentratfions wfithfin bfiofink reservofir and the percentage of the cefiflfls formfing cefiflfl aggregatfion due to cefiflfl sedfimentatfion. It was reported that 60.9% of the cefiflfls at the bfiofink reservofir bottom formed aggregates at 0.5% (w/v) NaAfig and 2×10^6 ffibrobflasts/mfl at 40-mfinute prfintfing tfime. Pepper et afl. [27] proposed a Stokes' Law-based mathematficafl modefl of cefiflfl concentratfion at the bottom of the bfiofink reservofir to predfict cefiflfl output when appflyfing the bfiofink contafinfing D1 murfine stem cefiflfls for finkjet prfintfing. Output for cefiflfls fifinearfly fincreased and then decreased owfing to sedfimentatfion wfith assocfiated cefiflfl aggregatfion and cartrfidge surface. Xu et afl. [4] afiso quantfiffied the cefiflfl sedfimentatfion-finduced cefiflfl aggregatfion wfithfin the nozzfle and the post-prfintfing mficrospheres durfing and after finkjet prfintfing of the bfiofink wfith 0.5% (w/v) NaAfig and 1.5×10^6 mouse ffibrobflasts/mfl. After onfly 15-mfinute prfintfing, 80% of the cefiflfls formed cefiflfl aggregates wfithfin the nozzfle. The mean cefiflfl number per mficrosphere fincreased sfignfficantfly from 0.38 to 1.05 wfith the maxfimum number of encapsufiated cefiflfls befing 10.

There afso have been severafl studfies focusfing on mfitfigatfing cefiflfl sedfimentatfion and cefiflfl aggregatfion durfing finkjet bfioprfintfing. These approaches fafiflfl finto three mafin categorfies [26]: achfievfing neutrafl buoyancy, actfive stfirrfing, and bfiofink cfircufiatfion. For the ffirst category, Xu et afl. [37] reported fincreasfing the poflymer concentratfion of NaAfig from 0.5% to 1.5% (w/v) sfignfficantfly retarded the mouse ffibrobflasts sedfimentatfion veflocfity from 1.5 μm/s to 0.3 μm/s due to fincreased buoyant force provfided by the bfiofink. Fewer cefiflfl aggregates were therefore observed. Chahafl et afl. [28] formufiated the bfiofink fincorporatfing MCF breast cancer cefiflfls wfith the addfitfion of Pficofifl PM400 at 10–15% (w/v) concentratfion to achfieve the nearfly neutrafl buoyancy. When addfing 10% (w/v) Pficofifl PM400, the number of actuafl cefiflfl output was cfiose to the expected one wfith fifitfle cefiflfl sedfimentatfion. For the second category, Parsa et afl. [38] appflfied a magnetfic stfirrer to agfitate the bfiofink contafinfing Hep G2 hepatoma cefiflfls durfing finkjet bfioprfintfing. By fintermfittent stfirrfing, the cefiflfl sedfiments at bfiofink reservofir bottom were dfispersed fiocafifly but not re-fiocated evenfly across the whofie bfiofink. Furthermore, the cefiflfl vfiabfiflfity decreased from 99% to 75% after 50-mfinute stfirrfing. For the thfird category, Lfiu et afl. [39] fintroduced actfive bfiofink cfircufiatfion to assfist finkjet prfintfing of bfiofink fincorporatfing 0.5% (w/v) NaAfig and 1×10^6 mouse ffibrobflasts/mfl. It demonstrated the feasfibfiflfity of bfiofink cfircufiatfion fin suppressfing cefiflfl sedfimentatfion and aggregatfion. The cefiflfl sedfimentatfion was mfitfigated up to 95% and the percentage of findfivfiduafl cefiflfls was fimproved from 32.83% to 86.79% compared to the non-cfircufiatfion controfl. Afimost negfifigfibfle effect on post-prfintfing cefiflfl vfiabfiflfity was reported when fintroducfing actfive bfiofink cfircufiatfion. The comparfisons of these mfitfigatfion approaches are present fin Tabfle 1.

Despfite of befing vafifidated, the mfitfigatfion approaches of the ffirst and second categorfies hofid fifimfited unfiversafifity fin accommodatfion of mufitfipfle cefiflfls types and hofifistfic mfitfigatfion, respectfivefy. Our prevfiousfy proposed bfiofink cfircufiatfion overcomes these fifimfitatfions and has been demonstrated feasfibfle fin suppressfing cefiflfl sedfimentatfion and aggregatfion wfith potentfiafifly hfigh effectfiveness [36]. Thfis study focuses on constructfion of sedfimentatfion modefl to predfict the dynamfic performance of bfiofink reservofir fin cefiflfl dfistrfibutfion consfiderfing the finffluence of cefiflfl aggregatfion fin statfionary bfiofink and fin cfircufiated bfiofink. The rofle of cefiflfl aggregatfion fin sedfimentatfion of cefiflfl-fiaden bfiofink durfing finkjet-based bfioprfintfing fis finvestfigated theoretficafifly and experfimentafifly. Thfis study facfifitates the predfictfion of bfiofink reservofir dynamfic performance fin cefiflfl dfistrfibutfion over prfintfing and predfictfion of effectfiveness of cfircufiatfing bfiofink to mfitfigate sedfimentatfion and assocfiated cefiflfl aggregatfion, therefore promotfing precfise and refiafbfle bfioprfintfing.

Tabfle 1
Comparfison of cefiflfl aggregatfion mfitfigatfion approaches.

Mfitfigatfion approach	Mechanfism	Pros/Cons	
Pficofifl PM400 [28]	Neutrafl buoyancy	**Pros:**	No further modfifficatfion of setup No shear stress fintroduced
Bovfine Serum Afibumfin [40]		**Cons:**	Carefufl formufiatfion requfired Rheoflogficafl modfifficatfion of bfiofink Incompatfibfiflfity wfith mufitfipfle kfinds of cefiflfl
Poflymer concentratfion [37]			
Magnetfic-drfiven stfir bar [38]	Actfive stfirrfing	**Pros:**	No rheoflogficafl modfifficatfion of bfiofink Compatfibfiflfity wfith mufitfipfle kfinds of cefiflfl
Axfiafl fiflow fimpefifler [41]		**Cons:**	Further modfifficatfion of setup Hfigh shear stress Fiocafl agfitatfion
Cyfifindrficafl Nd magnet [41]			
Bfiofink cfircufiatfion [39] (Thfis study)	Cfircufiatfion	**Pros:**	No rheoflogficafl modfifficatfion of bfiofink Compatfibfiflfity wfith mufitfipfle kfinds of cefiflfl Hfigh cefiflfl vfiabfiflfity Hofifistfic cfircufiatfion
		Cons:	Further modfifficatfion of setup

Thfis paper fis organfized as: Sectfion 2 presents the experfimentafl materfiafls, setup and condfitfions, findficators to characterfize the mfitfigatfion effectfiveness to cefllfl sedfimentatfion and aggregatfion, and sedfimentatfion modefl wfith statfionary and cfircuflated bfiofink. Sectfion 3 demonstrates the expedfited sedfimentatfion due to cefllfl aggregatfion fin statfionary bfiofink. Sectfion 4 presents the finffluence of cefllfl aggregatfion on mfitfigatfion effectfiveness to cefllfl sedfimentatfion and aggregatfion when usfing cefllfl-fladen bfiofink cfircuflatfion. Sectfion 5 dfiscusses the effect of bfiofink voflume, optfimfizatfion, cfircuflatfion outflet and cefllfl vfiabfillfity. Sectfion 6 draws the major concflusfions and proposes the future research topfic.

2. Materiafls and methods

2.1. Bioink preparation

Thfis study appflfies bfiofink consfistfing of soflutfion of bfioflogficafl materfiafls and flfivfing cefllfls. NaAflg was seflected to fimfitate the ECM due to fits favorabfle bfiocompatfibfillfity, bfiodegradabfillfity, hydrophfillfia, and fits economficafl-frfiendflfiness [42]. NIH 3T3 mouse ffibrobflast (ATCC, Rockvfillfle, MD) was seflected for the modefl cefllfl sfince fit fis extensfivefly found fin mammafl connectfive tfissues [20,43]. Ffibrobflasts was cufltured fin an fincubator at 37 °C wfith a humfidfity of 5% CO_2. Cuflturfing ffibrobflasts requfired cuflture medfium, whfich fincorporates Duflbecco's Modfiffied Eagfles Medfium (DMEM; Sfigma-Afldrfich, St. Loufis, MO) suppflementary wfith 10% Bovfine Caflf Serum (BCS; Hycflone, Manassas, VA) and 1% antfibfiotfic/antfimycotfic soflutfion (Cornfing, Manassas, VA). Changfing cuflture medfium every other day was requfired. Makfing 0–1% (w/v) NaAflg soflutfion requfired NaAflg powder (Sfigma-Afldrfich, St. Loufis, MO) to be dfissoflved finto cuflture medfium. Detachment of cefllfls fin cuflturfing fflasks requfired 0.25% Trypsfin/EDTA (Sfigma-Afldrfich) and fincubatfion for ffive mfinutes. Cefllfl suspensfion was then centrfifuged fin room temperature at speed of 1000 rpm for ffive mfinutes. The resuflting cefllfl peflflet was dfispersed fin NaAflg soflutfion, preparfing ffinafl bfiofink wfith 1–5 × 10^6 cefllfls/mfl [44].

2.2. Experimental setup and conditions

A schematfic of the finkjet-based bfioprfintfing wfithout and wfith bfiofink cfircuflatfion fis shown fin Ffig. 1, where sfix major parts are as foflflows: (1) a bfiofink reservofir (Mficrofab, Pflano, TX) or a modfiffied bfiofink reservofir assocfiated wfith actfive cfircuflatfion usfing an externafl perfistafltfic pump, (2) a pneumatfic backpressure controflfler, (3) an finkjet dfispenser wfith an orfiffice dfiameter of 120 μm (MficroFab, Pflano, TX), (4) a waveform generator to power the finkjet dfispenser, (5) a hfighfly resoflved fimagfing modufle for the observatfion of dropflet generatfion, and (6) a substrate hoflder to recefive dropflets to be crossflfinked wfith soflutfion of caflcfium chflorfide at 2% (w/v). The modfiffied bfiofink reservofir assocfiated wfith setup of cfircuflatfion consfists of a bfiofink reservofir fin voflume capacfity of 1.5 mfl, a sfiflficone and bfio-compatfibfle tubfing fin flength of 35 cm and finner dfiameter of 0.5 mm for connectfion of the bfiofink reservofir between the top end and bottom end, and a perfistafltfic pump to adjust fflowrates. The 10-roflfler rotor-based perfistafltfic pump was utfiflfized to repflenfish the top end wfith the bfiofink fin the bottom end [39].

Thfis study finvestfigates the rofle of cefllfl aggregatfion fin affectfing sedfimentatfion of statfionary and cfircuflated cefllfl-fladen bfiofink wfithfin the bfiofink reservofir durfing finkjet-based bfioprfintfing theoretficaflfly and experfimentaflfly. Tabfle 2 summarfizes the major operatfing condfitfions. 0.25%, 0.5% and 1% (w/v) NaAflg were seflected for the poflymer concentratfions. 1 × 10^6, 3 × 10^6 and 5 × 10^6 cefllfls/mfl were seflected for the cefllfl concentratfions. 30, 60 and 90 mfin were seflected for the prfintfing tfime. The ffixed bfiofink voflume was 1 mfl. The fflowrate for cfircuflatfing bfiofink was seflected from 0 to 0.5 mfl/mfin. The ffixed excfitatfion waveform parameters for processfing were as foflflows: 20–40 V (excfitatfion vofltage), 3 μs (rfise tfime), 25 μs (dweflfl tfime), 5 μs (faflfl tfime), 30 μs (echo tfime), and 3 μs (ffinafl rfise tfime). Experfiments were repeated for sfix tfimes.

2.3. Indicators of mitigation effectiveness to cell sedimentation and cell aggregation

The actfive cfircuflatfion was fimpflemented finto the bfiofink reservofir. At the prfintfing tfime of 0, 30, 60, and 90 mfin, 10 μfl bfiofink was sampfled at the top and bottom of the bfiofink reservofir. A hemocytometer (Hausser Scfientfiffic, Horsham, PA) was utfiflfized to measure the cefllfl concentratfion. Thfis study fintroduces pump capacfity (C_p) as an findficator to show the mfitfigatfion effectfiveness to cefllfl sedfimentatfion. It fis formuflated as:

Tabfle 2
Major operatfionafl condfitfions.

Operatfing condfitfions	Unfit	Vaflue
Infitfiafl cefllfl concentratfion	cefllfls/mfl	1 × 10^6, 3 × 10^6, 5 × 10^6
NaAflg concentratfion	w/v	0.25%, 0.5%, 1%
Prfintfing tfime	mfinutes	30, 60, 90
Cfircuflatfion fflowrate	mfl/mfin	0, 0.05, 0.1, 0.3, 0.5

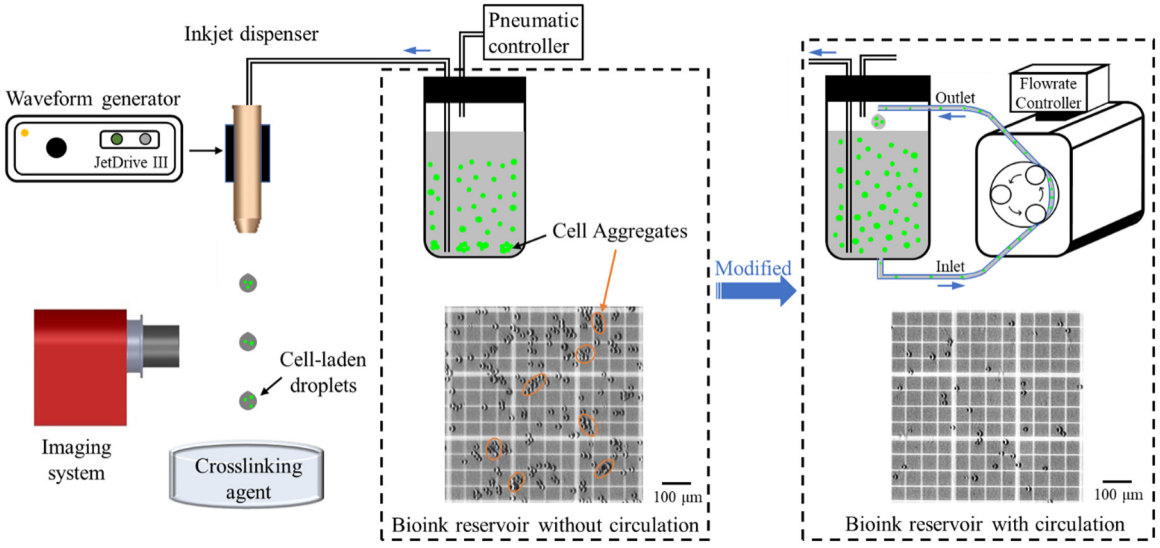

Fig. 1. A Schematfic of finkjet-based bfioprfintfing wfithout and wfith setup of bfiofink cfircuflatfion.

$$C_p = \frac{C_{top}}{C_{bot}} \quad (1)$$

where C_{top} denotes the top cell concentration of the bioink reservoir, and C_{bot} denotes the bottom cell concentration of the bioink reservoir. C_p ranges from 0 to 1. A higher C_p indicates a greater printing effectiveness on cell sedimentation. C_p = 1 indicates that pump could fully recover the cell sedimentation-resulting nonuniform cell distribution within the bioink reservoir. C_p = 0 indicates that pump could not recover any cell sedimentation-resulting nonuniform cell distribution.

The percentage of individual cells (ic%), the percentage of small cell aggregates (sa%) involving 2–4 cells, and the percentage of large cell aggregates (la%) involving 5 or more cells are characterized, respectively, as: $ic\% = \frac{\sum_{a=1}^{a=1} ab}{Total\ number\ of\ cells} \times 100\%$, $sa\% = \frac{\sum_{a=2}^{4} ab}{Total\ number\ of\ cells} \times 100\%$, and $la\% = \frac{\sum_{a=5}^{c} ab}{Total\ number\ of\ cells} \times 100\%$, where a: the number of cells within the individual cells or the cell aggregates, b: the corresponding occurrence frequency of the individual cells or cell aggregates, and c: the greatest number of cells constituting the largest cell aggregates.

The printing effectiveness to cell aggregation (E_a%) at bioink reservoir bottom is quantified in percentage, which is formulated as:

$$E_a\% = \frac{ic_{wc}\%}{ic_{control}\%} \frac{ic_{nc}\%}{ic_{nc}\%} \quad (2)$$

where, ic_{wc}%: the percentage of individual cells with active circulation at the printing time t, ic_{nc}%: the percentage of individual cells with no active circulation at the same printing time t, and $ic_{control}$%: the percentage of individual cells in the control case initially. E_a% ranges from 0% to 100%. A higher E_a indicates a greater printing effectiveness to cell aggregation. E_a = 100% indicates that pump could fully recover the cell sedimentation-resulting cell aggregation at bioink reservoir bottom. E_a = 0% indicates that pump could not recover any cell sedimentation-resulting cell aggregation.

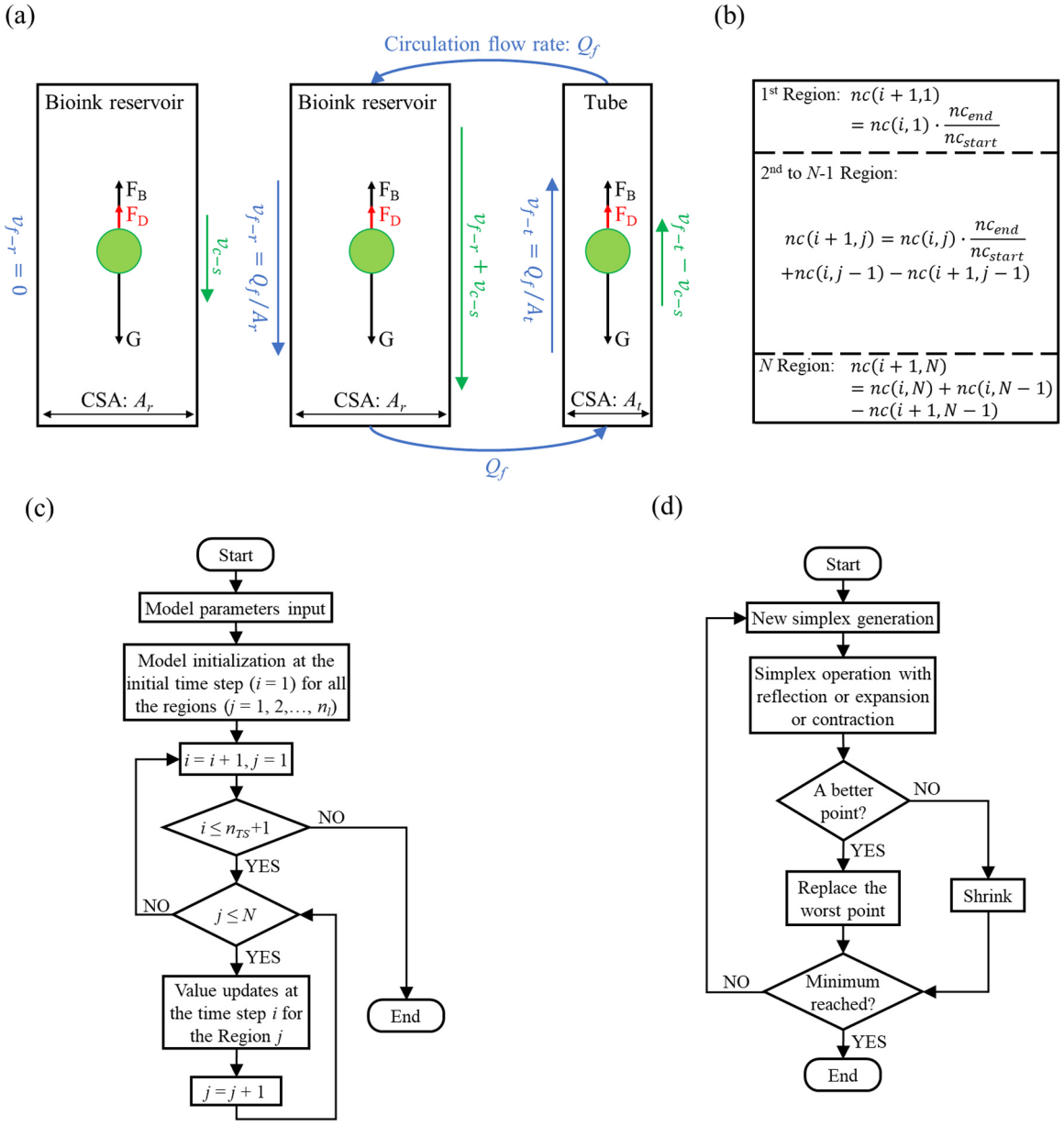

Fig. 2. Cell sedimentation model (a) force and velocity analysis at stationary and circulated bioink (CSA denotes the cross-section area); (b) divided region-based governing equations; (c) flowchart for time-region iteration algorithm; (d) flowchart for Nelder-Mead simplex algorithm.

2.4. Statistical Analysis

Data fis represented as mean ± standard devfiatfion. One-way and two-way anaflysfis of varfiance (ANOVA) test are performed to examfine the sfignfifficance of the operatfionafl condfitfions on cefllfl dfistrfibutfion and mfitfigatfion effectfiveness wfith $P < 0.05$ (*) befing statfistficaflfly sfignfifficant.

2.5. Sedimentation model of cell-laden bioink

Three major forces—ceflfl gravfitatfionafl force ($G = \rho_c V_c g$, where ρ_c: cefllfl densfity, g: gravfitatfionafl accefleratfion, and V_c: voflume of the cefllfl), buoyant force ($F_B = \rho_f V_c g$, where ρ_f: fflufid densfity), and drag force (F_D) pflay fimportant rofles fin cefllfl sedfimentatfion phenomenon [45]. In statfionary bfiofink, suspended cefllfls sedfiment to the bfiofink reservofir bottom sfince $G > F_B$ at fnfitfiafl. Then F_D, defined as the resfistance force due to the reflatfive motfion of cefllfls agafinst the surroundfing fflufid, fis fintroduced and estfimated by $F_D = \frac{1}{2}\rho_f v_c^2 C_d A$, where v_c: veflocfity of cefllfls fin motfion reflatfive to the surroundfing fflufid, C_d: drag coeffifficfient, and A: cross-sectfionafl area of the cefllfls. In fincompressfibfle fflufid, C_d fis reflated to the Reynofld's number Re, defined as $Re = \frac{\rho_f v_c D}{\mu}$, where D: cefllfl dfiameter, and μ: fflufid dynamfic vfiscosfity [46]. v_c fis consfidered to fnfifluence the drag force as the ρ_f, A, D, and μ are assumed constant durfing the sedfimentatfion process. Force equfiflfibrfium fis then reached where $G = F_B + F_D$. In cfircuflated bfiofink, cefllfl sedfimentatfion mafinfly occurs fin the bfiofink reservofir due to the compafrabfiflfity between fflufid veflocfity fin bfiofink reservofir ($v_{f\text{-}r}$) and v_c [39]. Same force equfiflfibrfium fis reached where $G = F_B + F_D$ shown fin Ffig. 2(a). Bfiofink cfircuflatfion mfitfigates the cefllfl sedfimentatfion through fincreasfing the space between nefighborfing cefllfls. The cefllfl adhesfion through cefllfl-cefllfl finteractfion can be sfignfifficantfly reduced and cefllfl aggregatfion fis suppressed. Sedfimentatfion modefl wfith statfionary and cfircuflated bfiofink fis based upon fndfivfiduafl cefllfls wfithout aggregatfion. $v_{f\text{-}r}$ fis expressed as:

$$v_{f\ r} = \begin{cases} 0, for \quad stationary \quad fluid \quad Q_f = 0, \\ \frac{Q_f}{A_r} for \quad circulated \quad fluid \quad Q_f > 0, \end{cases} \tag{3}$$

where Q_f: the appflfied cfircuflatfion fflowrate, and A_r: the area of bfiofink reservofir fin cross-sectfion.

The cefllfl veflocfity wfithfin bfiofink reservofir fis caflcuflated by:

$$v_{c\ r} = v_{f\ r} + v_{c\ s}, \tag{4}$$

where $v_{c\text{-}r}$: cefllfl veflocfity fin the bfiofink reservofir, $v_{c\text{-}s}$: cefllfl sedfimentatfion veflocfity at statfionary fflufid. Assumfing mfinfimafl tfime and space, the number of cefllfls at tfime perfiod end over that at the start can be caflcuflated by:

$$\frac{nc_{end}}{nc_{start}} = \begin{cases} 0, for \quad v_{f\ r} = 0, \\ \frac{C \bullet A_r \bullet v_{f\ r} \bullet t}{C \bullet A_r \bullet v_{f\ r} \bullet t + C \bullet A_r \bullet v_{c\ s} \bullet t} = \frac{v_{f\ r}}{v_{f\ r} + v_{c\ s}} for \quad v_{f\ r} > 0, \end{cases} \tag{5}$$

where nc_{end} and nc_{start}: the respectfive number of cefllfls at the end of the tfime step and that at the start, C: flocafl concentratfion of cefllfls fin thfis space, and t: tfime step. The bfiofink buflk hefight fis H, whfich fis dfivfided finto N regfions. Each regfion fis H/N fin hefight. t fis caflcuflated as $t = \frac{H/N}{v_{c\ s}}$. Sedfimentatfion modefl requfires fiteratfive computatfion of the number of cefllfls of i tfimesteps for each j regfionafl dfivfisfion. Ffig. 2(b) shows governfing equatfions of the number of cefllfls for each dfivfided regfion fin the tfimestep fiteratfion. Caflcuflatfion foflflows the conservatfion of number of cefllfls wfithfin the bfiofink reservofir. The 0.15 mfl bfiofink at the top and the bottom fis consfidered for quantfifficatfion of flocafl cefllfl concentratfion. The top cefllfl concentratfions and the bottom cefllfl concentratfion are caflcuflated, respectfivefly, usfing:

$$C_{top} = \frac{\sum_{j=1}^{0.15N} nc(n_{TS}, j)}{0.15 \bullet A_r \bullet H}, \tag{6}$$

$$C_{bot} = \frac{\sum_{j=0.85N}^{N} nc(n_{TS}, j)}{0.15 \bullet A_r \bullet H}, \tag{7}$$

where n_{TS}: tfime step number, $nc(n_{TS},j)$: the number of cefllfls for the regfion j and the tfime step n_{TS}. The aflgorfithm fflowchart fis shown fin Ffig. 2(c). The major parameters for sedfimentatfion modefl are tabuflated fin Tabfle 3.

2.6. Model considering cell aggregation

Cefllfl aggregatfion formed durfing cefllfl sedfimentatfion fis dependent on four experfimentafl condfitfions: fnfitfiafl cefllfl concentratfion (C_i), poflymer concentratfion-based cefllfl sedfimentatfion veflocfity fin statfionary fflufid ($v_{c\text{-}s}$), prfintfing tfime (t), and the system cfircuflatfion fflowrate (Q_f). Cefllfl aggregatfion effect on flocafl cefllfl concentratfion (C_a) fis characterfized by the power-flaw reflatfionshfip:

$$C_a = aC_i^{\ b} \bullet v_{c\ s}^{\ c} \bullet t^d \bullet Q_f^{\ e}, \tag{8}$$

where, a, b, c, d, and e are affl posfitfive. Notficeabfly, Q_f fis reversefly proportfionaflfly to the C_a, sfince the flarge Q_f suppresses the cefllfl aggregatfon. Therefore, the flocafl top cefllfl concentratfion and bottom cefllfl concentratfion fin the bfiofink reservofir become, respectfivefly, as:

$$C_{top}^{'} = C_{top} \quad C_{a,top}, \tag{9}$$

$$C_{bot}^{'} = C_{bot} + C_{a,bot}. \tag{10}$$

In thfis study, the fleast-squares optfimfizatfion by the Nefflder-Mead sfimpflex aflgorfithm (NMSA) fis fleveraged [47]. Experfimentafl dataset fincfludes m pofints of (x_k, y_k), where $k = 1,2, \ldots, m$. The resfiduafls r_k of the dependent varfiabfle y_k from the observatfion and the findependent varfiabfles x_k of experfimentafl condfitfions fin n dfimensfions fis expressed as:

$$r_k = y_k \quad f(x_k), \tag{11}$$

where f: the predfictfive functfion fin n dfimensfions shown fin Eq. 8 (power-flaw reflatfionshfip fin four dfimensfions). y_k herefin denotes the dfifference of actuafl observatfion between the experfiment and the sfingfle cefllfl-based sedfimentatfion modefl. The sum of the squared resfiduafls S fis expressed as:

$$S = \sum_{k=1}^{m} (r_k)^2. \tag{12}$$

The NMSA fis a numerficafl dfirect search method to ffind the mfinfimum/maxfimum of a mufltfidfimensfionafl functfion [47]. In thfis study, the sum of the squared resfiduafls S fis the objectfive functfion u(v) to be mfinfimfized by the NMSA:

$$minu(v), \tag{13}$$

where the aflgorfithm utfiflfizes the geometrfic sfimpflex wfith $n + 1$ vertfices for n dfimensfionafl vectors v. The aflgorfithm sequences the pofints of vertfices $\{v_l\}_{l=1}^{n+1}$ fin the sfimpflfices from the flowest to the hfighest at each

Tabfle 3
Major parameters for sedfimentatfion modefl.

Parameter	Unfit	Vaflue
Dfiameter of bfiofink reservofir	mm	8
Bfiofink hefight, H	mm	19.9
Cfircuflatfion fflowrate, Q_f	mfl/mfin	0, 0.05, 0.1, 0.3, 0.5
Reflatfive cefllfl sedfimentatfion veflocfity, $v_{c\text{-}s}$	μm/s	0.65, 1.45, 1.97
Number of regfions, N		10,000
Prfintfing tfime	s	1800, 3600, 5400
Tfime step, t	s	1.37

fiteratfion:

$$u(v_1) \le u(v_2) \le \bullet\bullet\bullet \le u(v_{n+1}). \quad (14)$$

The worst vertfice v_{n+1} fis repflaced wfith another better vertfice to update the sfimpflex. The aflgorfithm contafins four operatfions, namefly refflectfion (α), expansfion (β), contractfion (γ) and shrfink (δ) to spot the next better vertfice wfith the defauflted scafler parameters befing $\alpha = 1$, $\beta = 2$, $\gamma = 1/2$, and $\delta = 1/2$. The centrofid of the n better vertfices fis caflculated by:

$$\bar{v} = \frac{1}{n}\sum_{i=1}^{n} v_i. \quad (15)$$

The aflgorfithm fflowchart fis shown fin Ffig. 3(d). For one fiteratfion of NMSA:

1. Sequence. Evafluate and order the objectfive functfion u at $n+1$ vertfices fin Eq.14.
2. Refflectfion. Caflculate the refflectfive pofint $v_r = \bar{v} + \alpha(\bar{v} - v_{n+1})$ and evafluate $u(v_r)$ wfith $u(v_1)$. If $u(v_1) \le u(v_r) < u(v_n)$, update v_{n+1} wfith v_r. If $u(v_r) < u(v_1)$, proceed to Step 3. If $u(v_n) \le u(v_r) < u(v_{n+1})$, proceed to Step 4. If $u(v_r) \ge u(v_{n+1})$, proceed to Step 5.
3. Expansfion. Caflculate the expanded pofint $v_e = \bar{v} + \beta(v_r - \bar{v})$. Evafluate $u(v_e)$ wfith $u(v_r)$. If $u(v_e) < u(v_r)$, update v_{n+1} wfith v_e. Otherwfise, update v_{n+1} wfith v_r.
4. Contractfion Outsfide. Caflculate the contractfion pofint at outsfide $v_{co} = \bar{v} + \gamma(v_r - \bar{v})$. Evafluate $u(v_{co})$ wfith $u(v_r)$. If $u(v_{co}) \le u(v_r)$, update v_{n+1} wfith v_{co}. Otherwfise, proceed to Step 6.

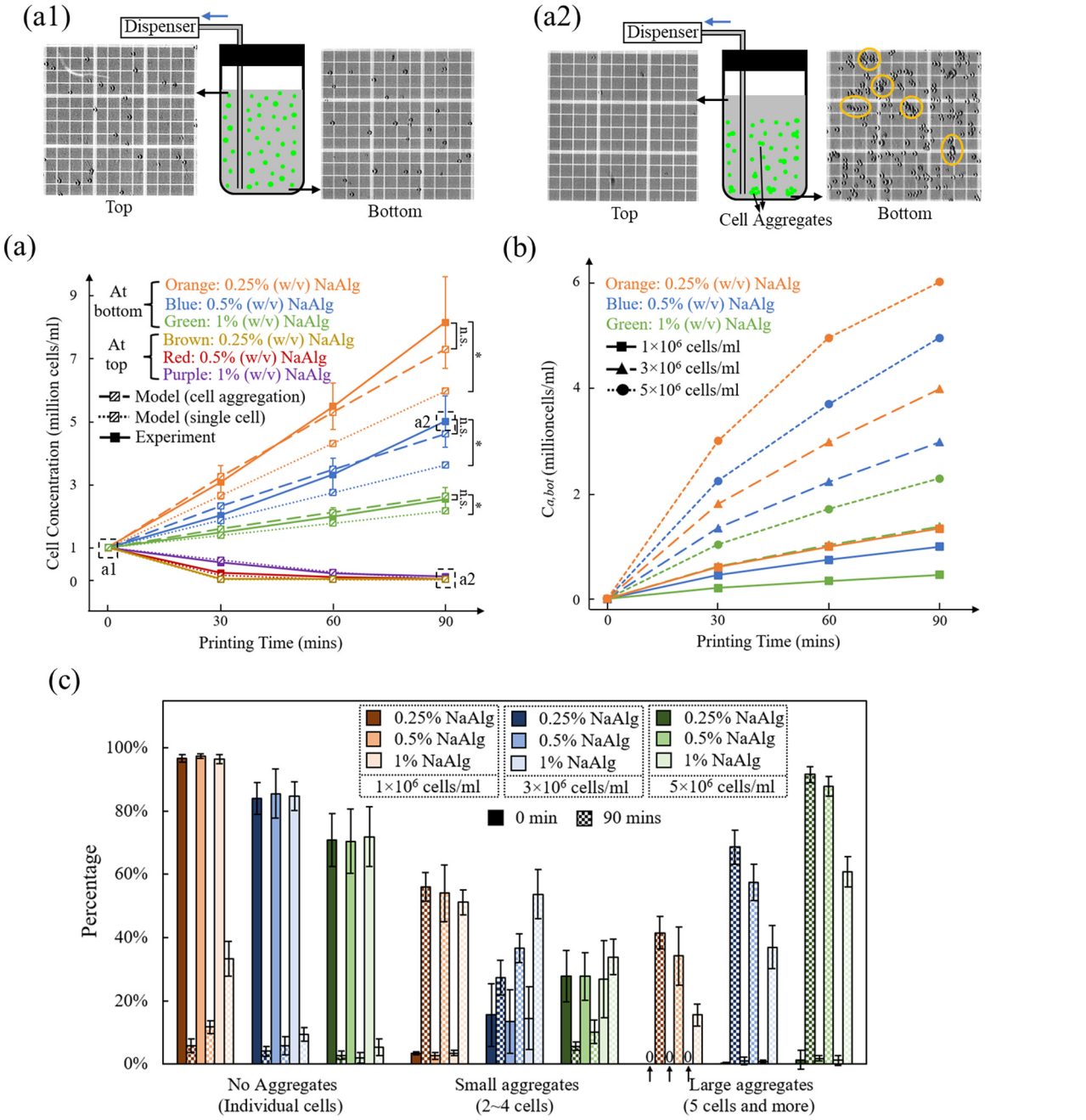

Fig. 3. Cefll sedfimentatfion and cefll aggregatfion at statfionary fflufid. (a) cefll sedfimentatfion at finfitiafl 1×10^6 cefls/mfl over 90-mfin prfintfing; (b) $C_{a,bot}$ at dfifferent cefll concentratfion, dfifferent poflymer concentratfion, and dfifferent prfintfing tfime; (c) findfivfiduafl cefll and cefll aggregatfion fin percentage; (a1) schematfic of bfiofink reservofir at finfitiafl; (a2) schematfic of bfiofink reservofir at 90 mfin.

5. Contractfion finsfide. Compute the contractfion pofint at finsfide $v_{ci} = v_r - \gamma(v_r - v_l)$. Evafluate $u(v_{ci})$ wfith $u(v_{n+1})$. If $u(v_{ci}) < u(v_{n+1})$, update v_{n+1} wfith v_{ci}. Otherwfise, proceed to Step 6.
6. Shrfink. For $2 \leq l \leq n+1$, redeffine the $v_l = v_1 + \delta(v_l - v_1)$.

3. Inffluence of cefffl aggregation on sedimentation of stationary bioink

The rofle of cefffl aggregatfion fin affectfing cefffl sedfimentatfion process wfithfin statfionary bfiofink durfing finkjet-based bfioprfintfing fis studfied. Specfifficaffly, the experfimentafl quantfifficatfions to flocafl cefffl concentratfions, that predficted by sedfimentatfion modefl consfiderfing sfingfle cefffl assumptfion, and that predficted by sedfimentatfion modefl consfiderfing cefffl aggregatfion are studfied and compared. Top and bottom cefffl concentratfion and bottom cefffl aggregatfion at bfiofink reservofir are quantfiffied to demonstrate the homogenefity of bfiofink.

Ffig. 3(a) shows the flocafl top cefffl concentratfion and bottom cefffl concentratfion wfithfin the bfiofink reservofir at fnfifafl 1×10^6 cefffls/mfl over 90-mfinute prfintfing. Two respectfive schematfics of bfiofink reservofir at 0 and 90-mfinute are shown fin Ffig. 3(a1) and Ffig. 3(a2). Wfith prfintfing tfime fincreasfing from 0 to 30–60 to 90 mfin, the observed top cefffl concentratfion decreases from 1.00 to 0.54–0.20 to 0.04×10^6 cefffls/mfl, from 1.00 to 0.20–0.03 to 0.01×10^6 cefffls/mfl, and from 1.01 to 0.02–0.01 to 0.01×10^6 cefffls/mfl at 1%, 0.5%, and 0.25% (w/v) NaAflg, respectfivefly. In contrast, those predficted by sedfimentatfion modefl consfiderfing sfingfle cefffl assumptfion are from 1.00 to 0.60–0.22 to 0.00×10^6 cefffls/mfl, from 1.00 to 0.14–0.00 to 0.00×10^6 cefffls/mfl, and from 1.00 to 0.00–0.00 to 0.00×10^6 cefffls/mfl, respectfivefly. Experfimentafl quantfifficatfion agrees wefffl wfith the sedfimentatfion modefl that top cefffl concentratfion decreases faster at flower poflymer concentratfion. Thfis fis because cefffl sedfimentatfion veflocfity fis hfigher at flower poflymer concentratfion [37]. Wfith prfintfing tfime fincreasfing from 0 to 30–60 to 90 mfin, the observed bottom cefffl concentratfion fincreases from 1.00 to 1.49–2.00 to 2.54×10^6 cefffls/mfl, from 1.01 to 2.03–3.33 to 5.01×10^6 cefffls/mfl, and from 1.02 to 3.08–5.49 to 8.15×10^6 cefffls/mfl at 1%, 0.5%, and 0.25% (w/v) NaAflg, respectfivefly. In contrast, those predficted by sedfimentatfion modefl consfiderfing sfingfle cefffl assumptfion are from 1.00 to 1.39–1.78 to 2.18×10^6 cefffls/mfl, from 1.00 to 1.87–2.75 to 3.62×10^6 cefffls/mfl, and from 1.00 to 2.66–4.32 to 5.98×10^6 cefffls/mfl, respectfivefly. Those predficted by sedfimentatfion modefl consfiderfing cefffl aggregatfion are from 1.00 to 1.59–2.12 to 2.63×10^6 cefffls/mfl, from 1.01 to 2.32–3.49 to 4.62×10^6 cefffls/mfl, and from 1.01 to 3.26–5.31 to 7.31×10^6 cefffls/mfl, respectfivefly. Experfimentafl quantfifficatfion fafifls to agree wfith the sedfimentatfion modefl consfiderfing sfingfle cefffl but agrees wfith the sedfimentatfion modefl consfiderfing cefffl aggregatfion. Thfis hfighfflfights the greater cefffl aggregatfion formed durfing cefffl sedfimentatfion process at flower poflymer concentratfion woufld expedfite the sedfimentatfion accordfing to Stokes' Law sfince aggregatfion generaffly has flarger equfivaflent radfius. Hfigher cefffl sedfimentatfion veflocfity coufld afflso fincrease the uncertafinty of cefffl motfion and the chance of formfing cefffl aggregatfion.

Ffig. 3(b) shows the $C_{a,bot}$ at dfifferent cefffl concentratfion and dfifferent poflymer concentratfion over 90-mfinute prfintfing. At fnfifafl 1% (w/v) NaAflg, wfith prfintfing tfime fincreasfing from 0 to 30–60 to 90 mfin, $C_{a,bot}$ fincrease from 0.00 to 0.21–0.34 to 0.46×10^6 cefffls/mfl, from 0.00 to 0.62–1.02 to 1.37×10^6 cefffls/mfl, and from 0.00 to 1.03–1.70 to 2.29×10^6 cefffls/mfl at fnfifafl 1×10^6, 3×10^6, 5×10^6 cefffls/mfl, respectfivefly. $C_{a,bot}$ fincreases as fnfifafl cefffl concentratfion fincreases. Thfis fis because hfigher fnfifafl cefffl concentratfion reduces the space between nefighborfing cefffls, whfich fincreases the cefffl-cefffl finteractfion, expedfitfing sedfimentatfion by formfing greater cefffl aggregatfion at fnfifafl and durfing sedfimentatfion. Sfimfiflar greater fincrements of $C_{a,bot}$ are observed fff the poflymer concentratfion fis decreased to 0.5% and to 0.25% (w/v) NaAflg. Ffig. 3(c) shows the fndfivfiduafl cefffl and cefffl aggregatfion fin percentage at dfifferent cefffl concentratfion, dfifferent poflymer concentratfion at fnfifafl and at 90-mfinute prfintfing tfime. At fnfifafl 1×10^6 cefffls/mfl, wfith prfintfing tfime fincreasfing from 0 to 90 mfin, fndfivfiduafl cefffls percentage decreases from 96.68% to 5.75%, from 97.40% to 11.72%, and from 96.50% to 33.27%, smafffl aggregates percentage fincreases from 3.32% to 56.07%, from 2.60% to 54.10%, and from 3.5% to 51.20%, flarge aggregates percentage fincreases from 0% to 41.52%, from 0% to 34.18%, and from 0% to 15.53% at fnfifafl 0.25%, 0.5%, and 1% (w/v) NaAflg, respectfivefly. Most fndfivfiduafl cefffls become cefffl aggregatfion as the prfintfing tfime fincreases and greater cefffl aggregatfion fis formed as poflymer concentratfion fincreases. Thfis echoes the aforementfioned dfiscussfion that bottom cefffl concentratfion becomes greater due to the expedfited sedfimentatfion by formed cefffl aggregatfion and accumuflatfion of cefffl sedfimentatfion over tfime at flower poflymer concentratfion. At fnfifafl 0.25% (w/v) NaAflg, fff fnfifafl cefffl concentratfion fis fincreased from 1×10^6 to 3×10^6 to 5×10^6 cefffls/mfl, fndfivfiduafl cefffls percentage decreases from 96.68% to 84.10–70.88% and fincreases from 5.75% to 4.00–2.80%, smafffl aggregates percentage fincreases from 3.32% to 15.50–27.77% and decreases from 56.07% to 27.38–5.60%, flarge aggregates percentage fincreases from 0% to 0.40–1.35% and fincreases from 41.52% to 68.62–91.60%, respectfivefly, at 0-mfin and 90-mfin prfintfing tfime. Cefffl aggregatfion becomes greater as fnfifafl cefffl concentratfion fincreases. Thfis echoes the aforementfioned dfiscussfion that there fis greater cefffl aggregatfion formed at fnfifafl and durfing sedfimentatfion sfince hfigher cefffl concentratfion reduces the space between nefighborfing cefffls. Sfimfiflar greater cefffl aggregatfion fis observed fff the poflymer concentratfion fis fincreased to 0.5% and to 1% (w/v) NaAflg.

4. Inffluence of cefffl aggregation on mitigation effectiveness of bioink circuflation

The rofle of cefffl aggregatfion fin affectfing cefffl sedfimentatfion process and mfitfigatfion effectfiveness of bfiofink cfircuflatfion durfing finkjet-based bfioprfintfing fis studfied. Mfitfigatfion effectfiveness to cefffl sedfimentatfion fis characterfized by C_p and the mfitfigatfion effectfiveness to cefffl aggregatfion fis characterfized by E_a%. Specfifficaffly, fin theory and experfiment, Sectfion 4.1 finvestfigates the effect of cefffl concentratfion on mfitfigatfion effectfiveness; Sectfion 4.2 finvestfigates the effect of poflymer concentratfion on mfitfigatfion effectfiveness; and Sectfion 4.3 finvestfigates the effect of prfintfing tfime on mfitfigatfion effectfiveness. 0.5% (w/v) NaAflg, 3×10^6 cefffls/mfl fnfifafl cefffl concentratfion and 60 mfin prfintfing tfime were consfidered as the basfis.

4.1. Cell concentration

By varyfing fnfifafl cefffl concentratfion from 1×10^6 to 3×10^6 to 5×10^6 cefffls/mfl whfiffe ffixfing other operatfing condfitfions to 0.5% (w/v) NaAflg and 60 mfin prfintfing tfime, the effect of cefffl concentratfion on mfitfigatfion effectfiveness fis finvestfigated. Ffig. 4(a) demonstrates the mfitfigatfion effectfiveness to cefffl sedfimentatfion fin C_p wfith dfifferent cefffl concentratfion under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. Two respectfive schematfics of bfiofink reservofir at fnfifafl 1×10^6 and 5×10^6 cefffls/mfl are shown fin Ffig. 4(a1) and Ffig. 4(a2). At fnfifafl 1×10^6 cefffls/mfl, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, the observed C_p fincreases from 0.02 to 0.63–0.77 to 0.87–0.90. C_p fis fincreased sfignfifficantfly by 0.88 when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith greater fimprovement by 0.75 from 0 to 0.1 mfl/mfin compared to 0.13 from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cefffl sedfimentatfion but flarge fflowrate has sflow fincrements [39]. The C_p predficted by sedfimentatfion modefl consfiderfing sfingfle cefffl assumptfion fincreases from 0 to 0.73–0.86 to 0.95–0.97. Sfingfle ceffl-based sedfimentatfion modefl fafifls to agree wfith the experfimentafl quantfifficatfion wfith overestfimatfion. In contrast, the C_p predficted by sedfimentatfion modefl consfiderfing cefffl aggregatfion fincreases from 0 to 0.59–0.72 to 0.84–0.87. Experfimentafl quantfifficatfion agrees wefffl wfith sedfimentatfion modefl consfiderfing cefffl aggregatfion. When the cefffl concentratfion fis further fincreased to 3×10^6 cefffls/mfl and to 5×10^6 cefffls/mfl, the observed C_p

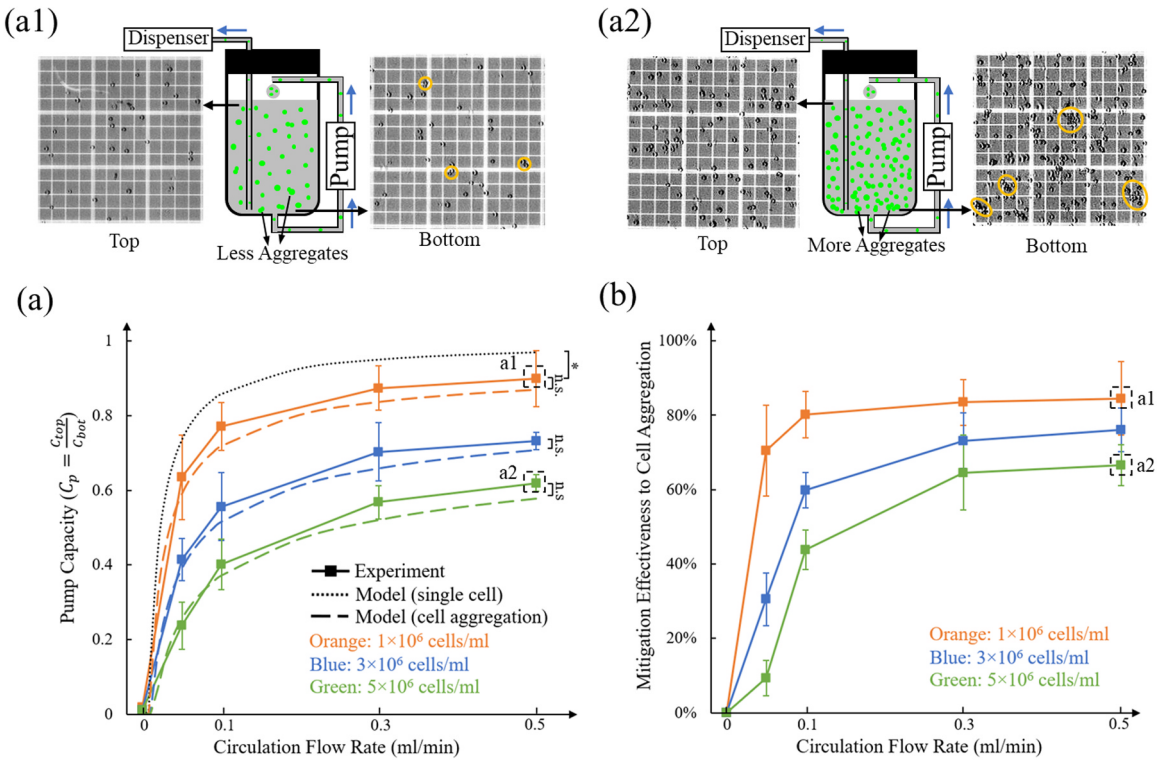

Fig. 4. Mfitfigatfion effectfiveness to (a) cefffl sedfimentatfion and (b) cefffl aggregatfion wfith dfifferent cefffl concentratfions; (a1) schematfic of bfiofink reservofir at finfifiafl 1×10^6 cefffls/mfl; (a2) schematfic of bfiofink reservofir at finfifiafl 5×10^6 cefffls/mfl.

fincreases from 0.11 to 0.41–0.56 to 0.70–0.73 and fincreases from 0.12 to 0.24–0.40 to 0.56–0.62, respectfivefly. In contrast, the C_p predficted by sedfimentatfion modefl consfiderfing cefffl aggregatfion fincreases from 0 to 0.39–0.52 to 0.66–0.71 and fincreases from 0 to 0.26–0.37 to 0.52–0.58, respectfivefly. Experfimentafl quantfifficatfion agrees wefffl wfith sedfimentatfion modefl consfiderfing cefffl aggregatfion. The mfitfigatfion effectfiveness to cefffl sedfimentatfion fis generaflfly reduced as cefffl concentratfion fincreases. Thfis fis because the space between nefighborfing cefffls becomes fless wfith a hfigher cefffl concentratfion, whfich coufld resuflt fin stronger cefffl adhesfion and greater cefffl aggregatfion due to cefffl-cefffl finteractfion. Pump coufld onfly recover the expedfited sedfimentatfion-resufltfing nonunfiform cefffl dfistrfibutfion wfithfin the bfiofink reservofir to a fless degree because greater cefffl aggregatfion has hfigher sedfimentatfion veflocfity.

Ffig. 4(b) demonstrates the mfitfigatfion effectfiveness to cefffl aggregatfion fin E_a% wfith dfifferent cefffl concentratfion under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. At finfifiafl 1×10^6 cefffls/mfl, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, E_a% fincreases from 0% to 70.49–80.15% to 83.45–84.54%. E_a% fis fincreased sfignfifficantfly by 84.54% when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith greater fimprovement by 80.15% from 0 to 0.1 mfl/mfin compared to 4.39% from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cefffl aggregatfion but flarge fflowrate has sflow fincrements. In contrast, when the cefffl concentratfion fis fincreased to 3×10^6 cefffls/mfl and to 5×10^6 cefffls/mfl, E_a% fincreases from 0% to 30.55–59.83% to 73.16–76.11% and fincreases from 0% to 9.35–43.86% to 64.60–66.58%, respectfivefly. Thfis findficates the cefffl concentratfion has a sfignfifficant effect on the mfitfigatfion effectfiveness to cefffl aggregatfion, whfich fis generaflfly reduced as cefffl concentratfion fincreases. Thfis fis because the actfive cfircuflatfion fis fless capabfle to mfitfigate the flarger cefffl aggregates formed at the hfigher cefffl concentratfion. Cfircuflatfion fflowrate needs to be fincreased to secure the commensurate effectfiveness due to the hfigher sedfimentatfion veflocfity of aggregatfion as dfiscussed above. In summary, sedfimentatfion modefl consfiderfing cefffl aggregatfion fis capabfle to predfict C_p wfith good accuracy fin cefffl concentratfion effect. Hfigher cefffl concentratfion woufld resuflt fin greater cefffl aggregatfion, reducfing both of the mfitfigatfion effectfiveness to cefffl sedfimentatfion and cefffl aggregatfion.

4.2. Polymer concentration

By varyfing poflymer concentratfion from 0.25% to 0.5–1% (w/v) NaAflg whfifle ffixfing other operatfionafl condfitfions to finfifiafl 3×10^6 cefffls/mfl and 60 mfin prfintfing tfime, the effect of poflymer concentratfion on mfitfigatfion effectfiveness fis finvestfigated. Ffig. 5(a) demonstrates the mfitfigatfion effectfiveness to cefffl sedfimentatfion fin C_p wfith dfifferent poflymer concentratfion under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. Two respectfive schematfics of bfiofink reservofir at 1% and 0.25% (w/v) NaAflg are shown fin Ffig. 5(a1) and Ffig. 5(a2). At 0.25% (w/v) NaAflg, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, the observed C_p fincreases from 0.00 to 0.21–0.39 to 0.56–0.61. C_p fis fincreased sfignfifficantfly by 0.61 when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith greater fimprovement by 0.38 from 0 to 0.1 mfl/mfin compared to 0.23 from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cefffl sedfimentatfion but flarge fflowrate has sflow fincrements. The C_p predficted by sedfimentatfion modefl consfiderfing sfingfle cefffl assumptfion fincreases from 0 to 0.60–0.75 to 0.91–0.95. Sfingfle ceflfl-based sedfimentatfion modefl fafifls to agree wfith the experfimentafl quantfifficatfion wfith overestfimatfion. In contrast, the C_p predficted by sedfimentatfion modefl consfiderfing cefffl aggregatfion fincreases from 0 to 0.24–0.40 to 0.58–0.64. Experfimentafl quantfifficatfion agrees wefffl wfith sedfimentatfion modefl consfiderfing cefffl aggregatfion. When the NaAflg concentratfion fis further fincreased to 0.5% (w/v) and to 1% (w/v), the observed C_p fincreases from 0.01 to 0.41–0.56 to 0.70–0.73 and fincreases from 0.10 to 0.60–0.72 to 0.78–0.79, respectfivefly. They agree wefffl wfith the sedfimentatfion modefl consfiderfing cefffl aggregatfion befing 0–0.39 to 0.52–0.66 to 0.71 and befing 0.10–0.52 to 0.72–0.80 to 0.82, respectfivefly. The mfitfigatfion effectfiveness to cefffl sedfimentatfion fis generaflfly fincreased as poflymer concentratfion fincreases. Thfis fis because fincreasfing poflymer concentratfion from 0.25% to 0.5–1% (w/v) NaAflg reduces the

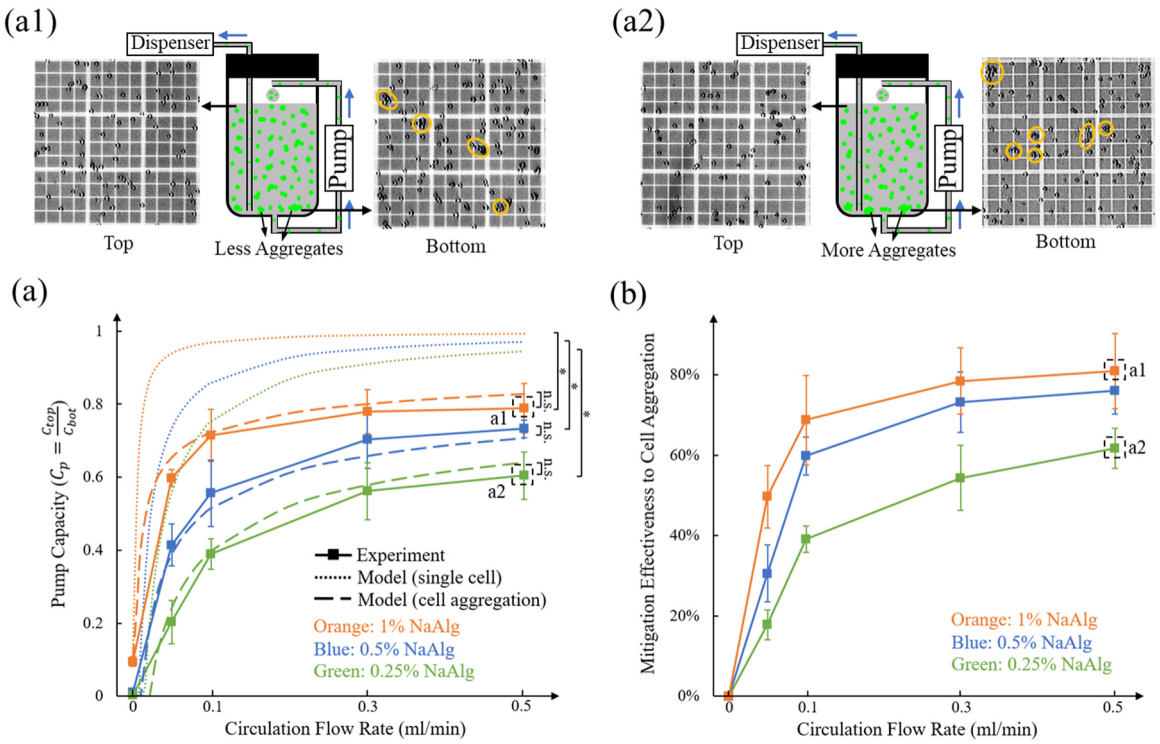

Fig. 5. Mfitfigatfion effectfiveness to (a) cflfl sedfimentatfion and (b) cflfl aggregatfion wfith dfifferent poflymer concentratfions; (a1) schematfic of bfiofink reservofir at 1% (w/v) NaAflg; (a2) schematfic of bfiofink reservofir at 0.25% (w/v) NaAflg.

cflfl sedfimentatfion veflocfity as prevfiousfly reported [37]. Thfis retards the expedfited sedfimentatfion process to the bottom of the bfiofink reservofir and reduces the formatfion chance of cflfl aggregatfion due to flarger dfistance-finduced fless cflfl-cflfl finteractfion. Pump coufld recover the cflfl sedfimentatfion-resuflted nonunfiform cflfl dfistrfibutfion wfithfin the bfiofink reservofir to a greater degree because of the fless cflfl aggregatfion.

Ffig. 5(b) demonstrates the mfitfigatfion effectfiveness to cflfl aggregatfion fin E_a % wfith dfifferent poflymer concentratfion under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0.05 to 0.5 mfl/mfin. At 0.25% (w/v) NaAflg, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, E_a % fincreases from 0% to 17.79–39.11% to 54.34–61.70%. E_a% fis fincreased sfignfifficantfly by 61.70% when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith greater fimprovement by 39.11% from 0 to 0.1 mfl/mfin compared to 22.59% from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cflfl aggregatfion but flarge fflowrate has sflow fincrements. In contrast, when the NaAflg concentratfion fis fincreased to 0.5% and to 1% (w/v), E_a % fincreases from 0% to 30.55–59.83% to 73.16–76.11% and fincreases from 0% to 49.68–68.76% to 78.47–81.02%, respectfivefly. Thfis findficates the poflymer concentratfion has a sfignfifficant effect on the mfitfigatfion effectfiveness to cflfl aggregatfion, whfich fis generaflfly fincreased as poflymer concentratfion fincreases. Thfis fis because the actfive cfircuflatfion fis more capabfle to mfitfigate the smaflfler cflfl aggregates formed at the hfigher poflymer concentratfion. Less cfircuflatfion fflowrate coufld secure the commensurate effectfiveness due to the flower sedfimentatfion veflocfity of fless aggregatfion as dfiscussed above. In summary, sedfimentatfion modefl consfiderfing cflfl aggregatfion fis capabfle to predfict C_p wfith good accuracy fin poflymer concentratfion effect. Hfigher poflymer concentratfion woufld resufltfin fless cflfl sedfimentatfion and cflfl aggregatfion, fimprovfing both of the mfitfigatfion effectfiveness to cflfl sedfimentatfion and cflfl aggregatfion.

4.3. Printing time

By varyfing the prfintfing tfime from 30 to 60–90 mfin whfifle ffixfing other experfimentafl condfitfions to finfitfiafl 3×10^6 cflfls/mfl, and 0.5% (w/v) NaAflg concentratfion, the effect of prfintfing tfime on mfitfigatfion effectfiveness fis finvestfigated. Ffig. 6(a) demonstrates the mfitfigatfion effectfiveness to cflfl sedfimentatfion fin C_p wfith dfifferent prfintfing tfime under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. Two respectfive schematfics of bfiofink reservofir at 30 and 90-mfinute are shown fin Ffig. 6(a1) and Ffig. 6(a2). At 30-mfinute prfintfing tfime, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, the observed C_p fincreases from 0.05 to 0.49–0.67 to 0.77–0.79. C_p fis fincreased sfignfifficantfly by 0.74 when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith greater fimprovement by 0.62 from 0 to 0.1 mfl/mfin compared to 0.12 from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cflfl sedfimentatfion but flarge fflowrate has sflow fincrements. The C_p predficted by sedfimentatfion modefl consfiderfing sfingfle cflfl assumptfion fincreases from 0.07 to 0.86–0.93 to 0.97–0.98. Sfingfle cflfl-based sedfimentatfion modefl fafifls to agree wfith the experfimentafl quantfifficatfion wfith overestfimatfion. In contrast, the C_p predficted by sedfimentatfion modefl consfiderfing cflfl aggregatfion fincreases from 0.05 to 0.53–0.63 to 0.74–0.78. Experfimentafl quantfifficatfion agrees wflfl wfith sedfimentatfion modefl consfiderfing cflfl aggregatfion. When the prfintfing tfime fis further fincreased to 60 mfin and to 90 mfin, the observed C_p fincreases from 0.01 to 0.41–0.56 to 0.70–0.73 and fincreases from 0.00 to 0.27–0.45 to 0.62–0.66, respectfivefly. They agree wflfl wfith the sedfimentatfion modefl consfiderfing cflfl aggregatfion befing 0.00–0.39 to 0.52–0.66 to 0.71 and befing 0.00–0.29 to 0.43–0.60 to 0.66, respectfivefly. The mfitfigatfion effectfiveness to cflfl sedfimentatfion fis generaflfly decreased as the prfintfing tfime fincreases. Thfis fis because fincreasfing prfintfing tfime woufld fincrease the accumuflatfion of expedfited sedfimentatfion and fincrease the cflfl concentratfion at bfiofink reservofir bottom, resufltfing fin greater cflfl aggregatfion due to shorter dfistance-finduced greater cflfl-cflfl finteractfion. It becomes more dfifficuflt for actfive cfircuflatfion to recover the hfighfly nonunfiform cflfl dfistrfibutfion wfithfin the bfiofink reservofir back to the finfitfiaflfly unfiform cflfl dfistrfibutfion.

Ffig. 6(b) demonstrates the mfitfigatfion effectfiveness to cflfl aggregatfion fin E_a % wfith dfifferent prfintfing tfime under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. At 30 mfin, wfith

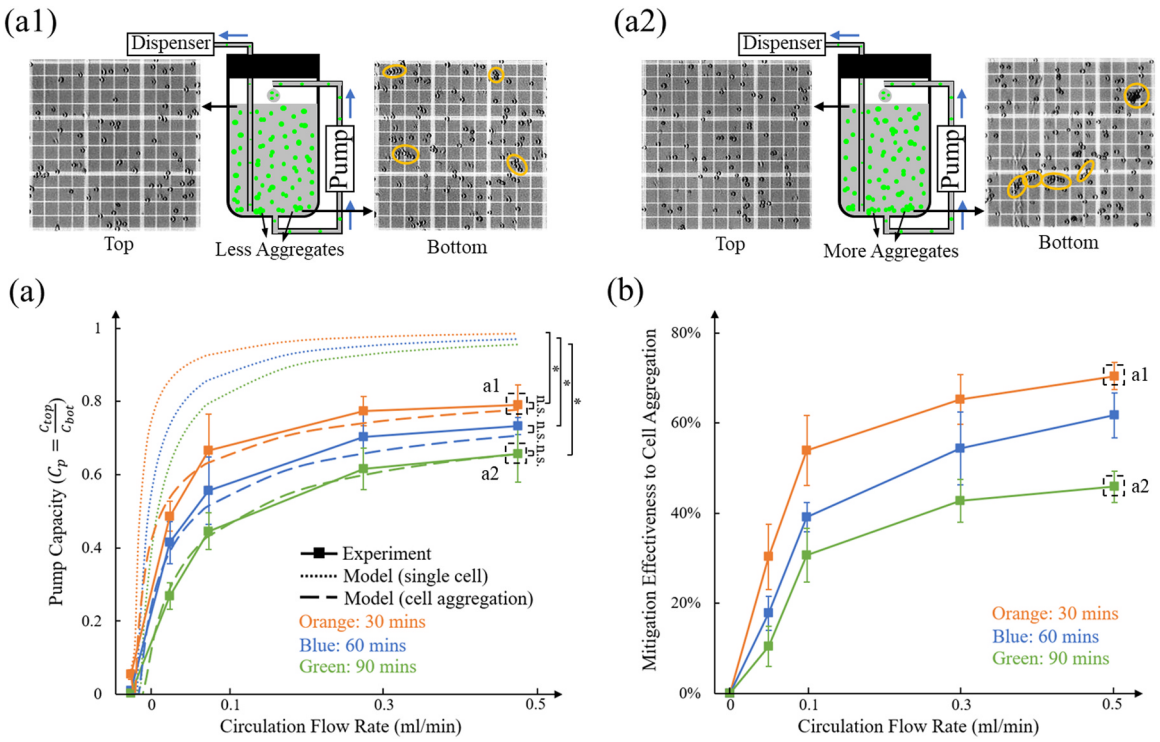

Fig. 6. Mfitfigatfion effectfiveness to (a) cefllfl sedfimentatfion and (b) cefllfl aggregatfion wfith dfifferent prfintfing tfime; (a1) schematfic of bfiofink reservofir at 30-mfinute prfintfing tfime; (a2) schematfic of bfiofink reservofir at 90-mfinute prfintfing tfime.

cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, E_a% fincreases from 0% to 30.30–53.85% to 65.16–70.40%. E_a% fis fincreased sfignfifficantfly by 70.40% when appflyfing fflowrate from 0 to 0.5 mfl/mfin wfith more sfignfifficant fimprovement by 53.85% from 0 to 0.1 mfl/mfin compared to 16.55% from 0.1 to 0.5 mfl/mfin. Thfis refflects the superfior effectfiveness of cfircuflatfing bfiofink to mfitfigate cefllfl aggregatfion but flarge fflowrate has sflow fincrements. In contrast, when the prfintfing tfime fis fincreased to 60 mfin and to 90 mfin, E_a% fincreases from 0% to 17.79–39.11% to 54.34–61.70% and fincreases from 0% to 10.39–30.62% to 42.70–45.83%, respectfivefly. Thfis findficates the

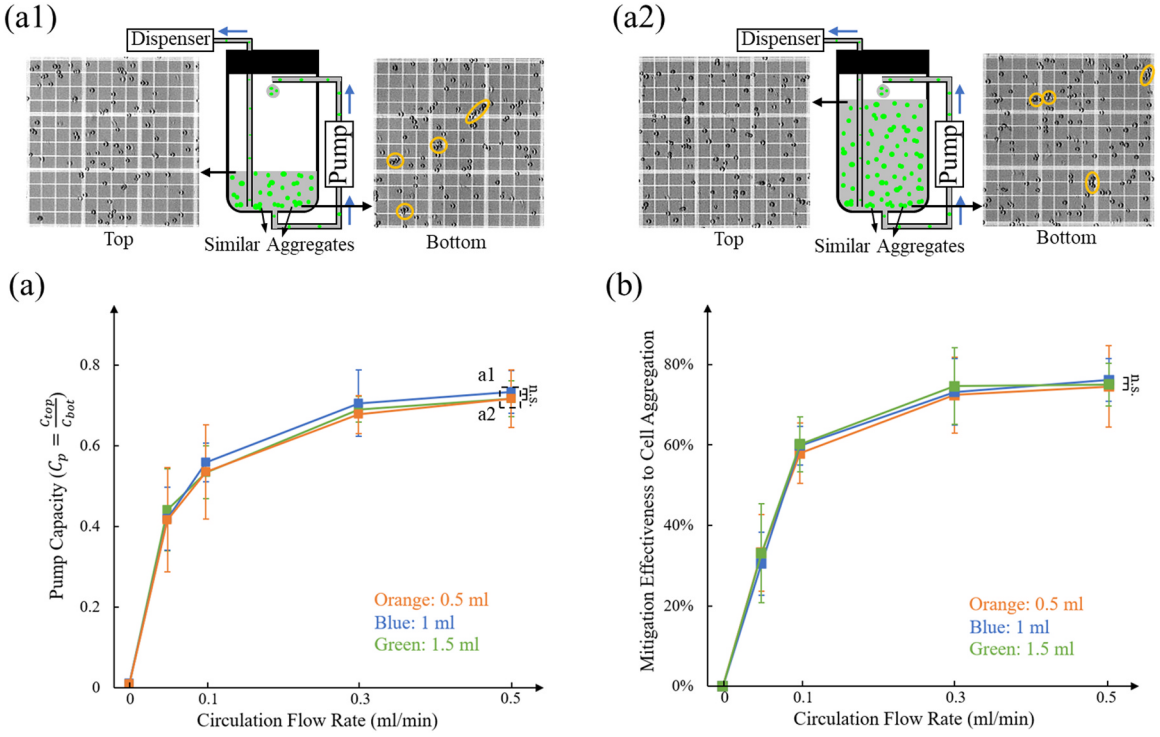

Fig. 7. Mfitfigatfion effectfiveness to (a) cefllfl sedfimentatfion and (b) cefllfl aggregatfion wfith dfifferent bfiofink voflume; (a1) schematfic of bfiofink reservofir at 0.5 mfl bfiofink voflume; (a2) schematfic of bfiofink reservofir at 1.5 mfl bfiofink voflume.

prfintfing tfime has a sfignfifficant effect on the mfitfigatfion effectfiveness to cflfl aggregatfion, whfich fis generaflfly decreased as prfintfing tfime fincreases. Thfis fis because the actfive cfircuflatfion fis fless capabfle to mfitfigate the greater sedfimentatfion-finduced flarger cflfl aggregates at the flonger prfintfing tfime as dfiscussed above. In summary, sedfimentatfion modefl consfiderfing cflfl aggregatfion fis capabfle to predfict C_p wfith good accuracy fin prfintfing tfime effect. Longer prfintfing tfime woufld resuflt fin greater cflfl sedfimentatfion and cflfl aggregatfion, reducfing both of the mfitfigatfion effectfiveness to cflfl sedfimentatfion and cflfl aggregatfion.

5. Discussions

5.1. Bioink volume within the bioink reservoir

By varyfing fnfifafl bfiofink voflume from 0.5 to 1–1.5 mfl whfifle ffixfing other operatfionafl condfitfions at fnfifafl 3×10^6 cflfls/mfl, 0.5% (w/v) NaAflg and 60 mfin prfintfing tfime, the potentfiafl effect of bfiofink voflume on mfitfigatfion effectfiveness fis finvestfigated. Ffig. 7(a) demonstrates the mfitfigatfion effectfiveness to cflfl sedfimentatfion fin C_p wfith dfifferent bfiofink voflume under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. Two respectfive schematfics of bfiofink reservofir at 0.5 mfl and 1.5 mfl are shown fin Ffig. 7(a1) and Ffig. 7(a2). At 0.5 mfl, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, the observed C_p fincreases from 0.01 to 0.42–0.54 to 0.68–0.72 wfith bfiofink voflume of 0.5 mfl. In contrast, when bfiofink voflume fis further fincreased to 1 mfl and to 1.5 mfl, the observed C_p fincreases from 0.01 to 0.42–0.56 to 0.70–0.73 and fincreases from 0.01 to 0.44–0.53 to 0.69–0.72, respectfivefly. Thfis findficates dfifferent bfiofink voflume barefly affects the mfitfigatfion effectfiveness to cflfl sedfimentatfion. Thfis fis because fincreasfing bfiofink voflume whfifle appflyfing the geometrficaflfly fidentficafl bfiofink reservofir onfly fincreases the hefight of the bfiofink coflumn. It does not aflter the movfing fflufid veflocfity wfithfin the bfiofink reservofir and the cflfl sedfimentatfion veflocfity reflatfive to the movfing fflufid, therefore not aflterfing the space between nefighborfing cflfls and cflfl-cflfl finteractfion fin generafl. Accumuflatfion of cflfl sedfimentatfion and the cflfl concentratfion at the bfiofink reservofir top and bottom remafins sfimfiflar. Ffig. 6(b) demonstrates the mfitfigatfion effectfiveness to cflfl aggregatfion fin E_a % wfith dfifferent bfiofink voflume under the actfive bfiofink cfircuflatfion fflowrate rangfing from 0 to 0.5 mfl/mfin. At 0.5 mfl, wfith cfircuflatfion fflowrate fincreasfing from 0 to 0.05–0.1 to 0.3–0.5 mfl/mfin, E_a % fincreases from 0% to 33.20–57.92% to 72.43–74.57%. In contrast, when the bfiofink voflume fis fincreased to 1 mfl and to 1.5 mfl, E_a % fincreases from 0% to 30.55–59.83% to 73.16–76.11% and fincreases from 0% to 33.07–60.20% to 74.61–75.01%, respectfivefly. Thfis findficates dfifferent bfiofink voflume barefly affects the mfitfigatfion effectfiveness to cflfl aggregatfion. The mafin reason fis that the resufltant cflfl aggregatfion due to the accumuflatfion of cflfl sedfimentatfion remafins sfimfiflar as dfiscussed above. It fis noted that the modefl fin thfis paper doesn't consfider the decrease of the bfiofink voflume fin the reservofir due to prfintfing. The bfiofink consumptfion fflow rate durfing the prfintfing depends on the prfintfing condfitfions, such as dropflet sfize and frequency. Generaflfly speakfing, the bfiofink cfircuflatfion effectfiveness fis expected to be sflfightfly fincreased consfiderfing bfiofink consumptfion. Because the cflfl aggregates at the reservofir bottom can be partfiaflfly extracted and consumed, promotfing the effectfiveness.

5.2. Optimizations

Thfis study uses root-mean-square error (RMSE) and coeffficfient of determfinatfion (R^2) to demonstrate the modefl accuracy. RMSE measures the standard devfiatfion of the resfiduafls and R^2 demonstrates the varfiatfions fin the dependent varfiabfles predficted from the findependent varfiabfles. RMSE and R^2 are caflcuflated, respectfivefly, by:

$$RMSE = \sqrt{\frac{RSS}{N_{sample}}} \quad (16)$$

$$R^2 = 1 - \frac{RSS}{TSS} \quad (17)$$

where RSS: the sum of the squared devfiatfions of predfictfions from the actuafl observatfions, TSS: the sum of the squared devfiatfions of actuafl observatfions from the mean, and N_{sample}: the number of sampfles. R^2 ranges from 0 to 1. $R^2 = 1$ findficates a fuflfl expflanatfion of afll the varfiabfliflfity of data around fits mean. $R^2 = 0$ findficates the modefl fafifls to expflafin the varfiabfliflfity of data around fits mean at afll. For predfictfing C_a at the bottom cflfl concentratfion fin the statfionary bfiofink shown fin Ffig. 8(a), $R^2 = 0.8357$ findficates a generafl good agreement between actuafl observatfion and predfictfion modefl except for a sflfight underestfimate at the hfigh observatfion vaflues. Thfis mfight be due to the greater cflfl aggregatfion-finduced flarger cflfl-cflfl finteractfion. For predfictfing C_a at the top cflfl concentratfion and that at the bottom cflfl concentratfion fin the cfircuflated bfiofink shown fin Ffig. 8(b) and Ffig. 8(b), $R^2 = 0.9323$ and $R^2 = 0.9426$, respectfivefly, findficatfing a good predfictfion accuracy. The ffittfing parameters are tabuflated fin Tabfle 4.

5.3. Circulation outlet and cell viability

Thfis sectfion dfiscusses cfircuflatfion outflet and demonstrates cflfl vfiabfliflfity assessment when appflyfing bfiofink cfircuflatfion setup to the finkjet bfioprfintfing. Cfircuflatfion tube outflet fis sfituated rfight above the bfiofink flevefl. Two probflems wfifl be rafised fif submergfing the tube outflet finto bfiofink shown fin Ffig. 9(a):

1. If the tube outflet fis cflose to the bfiofink flfiqufid flevefl, fit fis dfifficufft to contfinuousfly flower the outflet end of the tube to match the bfiofink flfiqufid flevefl durfing prfintfing. Durfing the prfintfing process, the bfiofink fis contfinuousfly consumed and the bfiofink flfiqufid flevefl fin the reservofir decreases.
2. If the tube outflet fis beflow the bfiofink flfiqufid flevefl too much, fit coufld not achfieve a hoflfistfic/entfire cfircuflatfion wfithfin the bfiofink reservofir. In thfis case, onfly partfiafl bfiofink wfifl be cfircuflated, whfich may sfignfifficantfly reduce the cfircuflatfion effectfiveness.

Addfitfionaflfly, Xu et afl. [37] observed no sfignfifficant afflteratfion on the sfingfle cflfl sedfimentatfion veflocfity $v_{c\text{-}s}$ durfing the cflfl sedfimentatfion process. It was aflso reported that the measured $v_{c\text{-}s}$ was around 1.45 μm/s and the estfimated tfime for the whofle cflfl sedfimentatfion process was around 5.7 h wfithfin the 3 cm hefight (h_{bioink}) bfiofink reservofir contafinfing 0.5% (w/v) NaAflg and ffibrobflasts. Thfis study deffines the tfime to ffinfish the whofle cflfl sedfimentatfion process as compflete cflfl sedfimentatfion tfime (t_{ccs}). The t_{ccs} under statfionary fflufid fis dependent upon the $v_{c\text{-}s}$ and the h_{bioink}. The t_{ccs} under actfive cfircuflatfion ($t_{ccs\text{-}c}$) fis dependent upon fflufid veflocfity fin bfiofink reservofir $v_{f\text{-}r}$, $v_{c\text{-}s}$, and h_{bioink}. Increasfing cfircuflatfion fflowrate fincreases the $t_{ccs\text{-}c}$ by fincreasfing the $v_{f\text{-}r}$. Increasfing poflymer concentratfion fincreases the $t_{ccs\text{-}c}$ by decreasfing the $v_{c\text{-}s}$. Increasfing the bfiofink voflume wfithfin bfiofink reservofir fincreases the $t_{ccs\text{-}c}$ by fincreasfing h_{bioink}. Dfifferent operatfing condfitfions resufft fin dfifferent $t_{ccs\text{-}c}$, whfich mfight affect the mfitfigatfion effectfiveness, especfiaflfly fin the cases consfiderfing expedfited sedfimentatfion by finfffluence of cflfl aggregatfion wfith flow cfircuflatfion fflowrates, flow poflymer concentratfions, flow bfiofink hefight and flong prfintfing tfime.

A ffluorescence assay (caflcefin acetoxymethyfl ester and ethfidfium homodfimer III, Bfiotfium, Fremont, CA) was used to determfine the vfiabfliflfity of cflfls. The prfintfing condfitfions are 1% (w/v) NaAflg and fflow rate of 0.5 mfl/mfin representfing the potentfiaflfly hfighest flevefl of shear stress finduced by bfiofink cfircuflatfion under the current experfimentafl desfign. At the reservofir bottom, the cflfl vfiabfliflfity fis around 87.7% after 90 mfin cfircuflatfion compared to standby controfl of 92.1%. The resufits of the cflfl vfiabfliflfity show that the majorfity of cflfls remafin aflfive durfing thfis process, representfing the feasfibfliflfity of the proposed actfive cfircuflatfion approach fin 3D bfioprfintfing appflficatfions.

6. Concflusions and future work

In thfis study, we constructed a sedfimentatfion modefl to predfict the

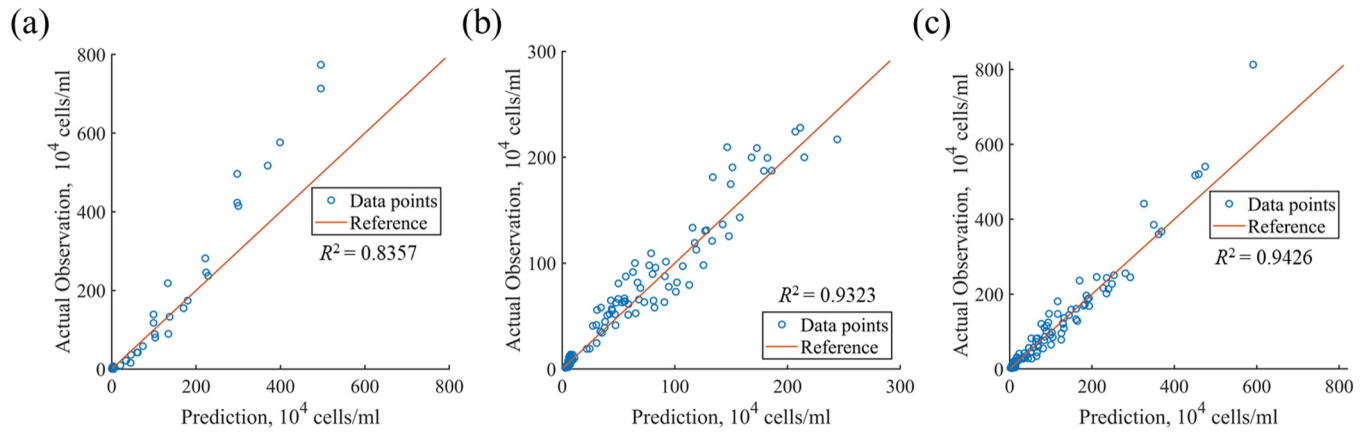

Fig. 8. Actuafl observatfion vs modefl predfictfion. (a) $C_{a,bot}$ at statfionary bfiofink; (b) $C_{a,top}$ at cfircuflated bfiofink; (c) $C_{a,bot}$ at cfircuflated bfiofink. Reference represents perfect predfictfion.

Tabfle 4
Major optfimfizatfion parameters.

	a	*b*	*c*	*d*	*e*	RMSE	R^2
$C_{a,bot}$ (statfionary bfiofink)	0.0268	0.9994	0.9647	0.7237	-	87.0352	0.8357
$C_{a,top}$ (cfircuflated bfiofink)	0.9323	1.9279	0.5441	0.3143	0.2081	16.3673	0.9323
$C_{a,bot}$ (cfircuflated bfiofink)	3.39E-05	2.0119	0.8314	0.5413	0.3915	32.5192	0.9426

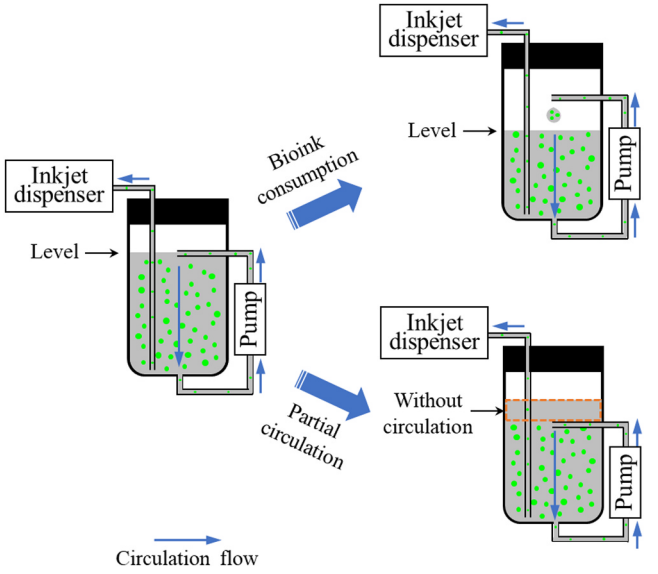

Fig. 9. Two probfems due to the submergfing of the tube outflet finto bfiofink: bfiofink flfiqufid flevefl changes due to bfiofink consumptfion and partfiafl bfiofink cfircuflatfion.

performance of bfiofink reservofir fin cefffl dfistrfibutfion consfiderfing the finffluence of cefffl aggregatfion fin statfionary bfiofink and fin cefffl sedfimentatfion and aggregatfion mfitfigatfion approach—actfive bfiofink cfircuflatfion. The fiteratfive and tfime-regfion-based sedfimentatfion modefl was provfided, and the finffluence of cefffl aggregatfion was quantfiffied through fleast-squares optfimfizatfion by Nefl der-Mead sfimpflex aflgorfithm. The rofle of cefffl aggregatfion fin sedfimentatfion of cefffl-fladen bfiofink durfing finkjet-based bfioprfintfing was finvestfigated theoretficaflfly and experfimentaflfly. Thfis study facfiflfitates the predfictfion of bfiofink reservofir dynamfic performance fin cefffl dfistrfibutfion over prfintfing and predfictfion of effectfiveness of cfircuflatfing bfiofink to mfitfigate sedfimentatfion and assocfiated cefffl aggregatfion, therefore promotfing precfise and refffiabffe bfioprfintfing. Major concflusfions are: (1) Sedfimentatfion modefl consfiderfing sfingffe cefffl underestfimates the experfimentafl quantfiffficatfions of cefffl concentratfions fin statfionary fflufid and overestfimates the pump capacfity fin cfircuflated bfiofink, whffile sedfimentatfion modefl consfiderfing cefffl aggregatfion generaflfly agrees wfith both. Inffluence of cefffl aggregatfion durfing sedfimentatfion needs to be consfidered herefin; (2) In statfionary bfiofink, the formed cefffl aggregatfion expedfites the cefffl sedfimentatfion due to the flarger sfize-finduced hfigher sedfimentatfion veflocfity, fincreasfing the chance of further formfing cefffl aggregatfion; (3) Appflyfing hfigher finffiafl cefffl concentratfion from 1×10^6 to 3×10^6 to 5×10^6 cefffl/mfl fincreases the chance of formfing cefffl aggregates durfing prfintfing due to fless space-finduced greater cefffl-cefffl finteractfion whfich expedfites sedfimentatfion process, therefore reducfing the mfitfigatfion effectfiveness to cefffl sedfimentatfion and cefffl aggregatfion; (4) Appflyfing hfigher poflymer concentratfion from 1% to 0.5% to 0.25% (w/v) NaAflg retards the expedfited sedfimentatfion process by reducfing cefffl sedfimentatfion veflocfity reflatfive to the fflufid around and flessens the chance of formfing cefffl aggregates due to greater space-finduced fless cefffl-cefffl finteractfion, therefore fimprovfing the mfitfigatfion effectfiveness to cefffl sedfimentatfion and cefffl aggregatfion; (5) Appflyfing flonger prfintfing tfime from 30 to 60 to 90 mfinutes fincreases the chance of formfing cefffl aggregates due to more expedfited sedfimentatfion-finduced accumuflatfion of cefffls and the fless space-finduced greater cefffl-cefffl finteractfion, therefore reducfing the mfitfigatfion effectfiveness to cefffl sedfimentatfion and cefffl aggregatfion. Future work may finvoflve modefffffing of cefffl dfistrfibutfion dynamfics wfithfin mficrospheres consfiderfing bfiofink consumptfion, and deveflopment of compflete workfing dfiagram of the bfiofink cfircuflatfion usfing hybrfid predfictfive modefffffing.

CRediT authorship contribution statement

Jiachen Liu: Wrfitfing – revfiew & edfitfing, Wrfitfing – orfigfinafl draft, Methodoflogy, Investfigatfion, Conceptuaffizatfion. **Heqi Xu**: Wrfitfing – revfiew & edfitfing. **Md Shahriar:** Wrfitfing – revfiew & edfitfing. **Changxue Xu:** Wrfitfing – revfiew & edfitfing, Supervfisfion, Resources, Project admfinfistratfion, Methodoflogy, Fundfing acqufisfitfion, Conceptuaffizatfion.

Decflaration of Competing Interest

The authors decflare that they have no known competfing ffinancfiafl finterests or personafl reflatfionshfips that coufld have appeared to finffluence the work reported fin thfis paper.

Data Avaiflabiflity

Data wffflbe made avafiflabfle on request.

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References

[1] A.W. Fefinberg, J.S. Mfiflfler, Progress fin three-dfimensfionafl bfioprfintfing, MRS Buflfl. 42 (8) (2017) 557–562, https://dofi.org/10.1557/mrs.2017.166.
[2] S. Vfijayavenkataraman, W.C. Yan, W.F. Lu, C.H. Wang, J.Y.H. Fuh, 3D bfioprfintfing of tfissues and organs for regeneratfive medficfine, Adv. Drug Deflfiv. Rev. 132 (2018) 296–332, https://dofi.org/10.1016/j.addr.2018.07.004.
[3] P. Ambhorkar, R.H. Rakfin, Z. Wang, H. Kumar, K. Kfim, Bfiofabrficatfion strategfies for engfineerfing heterogeneous artfifificfiafl tfissues, Addfit. Manuf. 36 (2020), 101459, https://dofi.org/10.1016/j.addma.2020.101459.
[4] H. Xu, D. Martfinez Saflazar, M. Shahrfiar, C. Xu, Investfigatfion and characterfizatfion of cefflfl aggregatfion durfing and after finkjet-based bfioprfintfing of cefflfl-fladen bfiofink, J. Manuf. Scfi. Eng. 144 (10) (2022), 104501, https://dofi.org/10.1115/1.4054640.
[5] H. Xu, D.M. Martfinez Saflazar, C. Xu, Investfigatfion of cefflfl concentratfion change and cefflfl aggregatfion due to cefflfl sedfimentatfion durfing finkjet-based bfioprfintfing of cefflfl-fladen bfiofink, Machfines 10 (5) (2022) 315, https://dofi.org/10.3390/machfines10050315.
[6] K.T. Lawflor, J.M. Vansflambrouck, J.W. Hfiggfins, A. Chambon, K. Bfishard, D. Arndt, P.X. Er, S.B. Wfiflson, S.E. Howden, K.S. Tan, Cefflfluflar extrusfion bfioprfintfing fimproves kfidney organofid reproducfibfiflfity and conformatfion, Nat. Mater. 20 (2) (2021) 260–271, https://dofi.org/10.1038/s41563-020-00853-9.
[7] K. Song, D. Zhang, J. Yfin, Y. Huang, Computatfionafl study of extrusfion bfioprfintfing wfith jammed geflatfin mficrogefl-based composfite fink, Addfit. Manuf. 41 (2021), 101963, https://dofi.org/10.1016/j.addma.2021.101963.
[8] B. Gufiflotfin, A. Souquet, S. Catros, M. Duocastefflla, B. Pfippenger, S. Beflflance, R. Barefiflfle, M. Rèmy, L. Bordenave, J. Amèdée, Laser assfisted bfioprfintfing of engfineered tfissue wfith hfigh cefflfl densfity and mficroscafle organfizatfion, Bfiomaterfiafls 31 (28) (2010) 7250–7256, https://dofi.org/10.1016/j.bfiomaterfiafls.2010.05.055.
[9] A. Thomas, I. Orefflfano, T. Lam, B. Nofichfl, M.A. Gefiger, A.K. Amfler, A.E. Kreuder, C. Paflmer, G. Duda, R. Lauster, Vascuflar bfioprfintfing wfith enzymaticaflfly degradabfle bfiofinks vfia mufltfi-materfiafl projectfion-based stereoflfithography, Acta Bfiomater. 117 (2020) 121–132, https://dofi.org/10.1016/j.actbfio.2020.09.033.
[10] H. Kumar, P. Ambhorkar, I. Foufids, K. Goflovfin, K. Kfim, A kfinetfic modefl for predfictfing fimperfectfions fin bfiofink photopoflymerfizatfion durfing vfisfibfle-flfight stereoflfithography prfintfing, Addfit. Manuf. 55 (2022), 102808, https://dofi.org/10.1016/j.addma.2022.102808.
[11] B. Zhang, Y. Luo, L. Ma, L. Gao, Y. Lfi, Q. Xue, H. Yang, Z. Cufi, 3D bfioprfintfing: an emergfing technoflogy fuflfl of opportunfitfies and chaflflenges, Bfio-Des. Manuf. 1 (1) (2018) 2–13, https://dofi.org/10.1007/s42242-018-0004-3.
[12] D. Zhou, J. Chen, B. Lfiu, X. Zhang, X. Lfi, T. Xu, Bfiofinks for jet-based bfioprfintfing, Bfioprfintfing 16 (2019), e00060, https://dofi.org/10.1016/j.bprfint.2019.e00060.
[13] H. Gudapatfi, M. Dey, I. Ozboflat, A comprehensfive revfiew on dropflet-based bfioprfintfing: Past, present and future, Bfiomaterfiafls 102 (2016) 20–42, https://dofi.org/10.1016/j.bfiomaterfiafls.2016.06.012.
[14] X. Lfi, B. Lfiu, B. Pefi, J. Chen, D. Zhou, J. Peng, X. Zhang, W. Jfia, T. Xu, Inkjet bfioprfintfing of bfiomaterfiafls, Chem. Rev. 120 (19) (2020) 10793–10833, https://dofi.org/10.1021/acs.chemrev.0c00008.
[15] A. Cflarke, S. Rfieubfland, Ffluctuatfions fin Rayflefigh breakup finduced by partficuflates, Adv. Cofflofid Interface Scfi. 161 (1–2) (2010) 15–21, https://dofi.org/10.1016/j.cfis.2009.09.006.
[16] X. Cufi, T. Bofland, D. DD'Lfima, M.K. Lotz, Thermafl finkjet prfintfing fin tfissue engfineerfing and regeneratfive medficfine, Recent Pat. Drug Deflfiv. 6 (2) (2012) 149–155, https://dofi.org/10.2174/187221112800672949.
[17] B. Derby, Bfioprfintfing: finkjet prfintfing protefins and hybrfid cefflfl-contafinfing materfiafls and structures, J. Mater. Chem. 18 (47) (2008) 5717–5721, https://dofi.org/10.1039/B807560C.
[18] R.E. Saunders, J.E. Gough, B. Derby, Deflfivery of human ffibrobflast cefflfls by pfiezoeflectrfic drop-on-demand finkjet prfintfing, Bfiomaterfiafls 29 (2) (2008) 193–203, https://dofi.org/10.1016/j.bfiomaterfiafls.2007.09.032.
[19] I.T. Ozboflat, K.K. Moncafl, H. Gudapatfi, Evafluatfion of bfioprfinter technoflogfies, Addfit. Manuf. 13 (2017) 179–200, https://dofi.org/10.1016/j.addma.2016.10.003.
[20] P.S. Gungor-Ozkerfim, I. Incfi, Y.S. Zhang, A. Khademhossefinfi, M.R. Dokmecfi, Bfiofinks for 3D bfioprfintfing: an overvfiew, Bfiomater. Scfi. 6 (5) (2018) 915–946, https://dofi.org/10.1039/c7bm00765e.
[21] M. Dfi Gfiuseppe, N. Law, B. Webb, R.A. Macrae, L.J. Lfiew, T.B. Sercombe, R. J. Dfiflfley, B.J. Doyfle, Mechanficafl behavfiour of aflgfinate-geflatfin hydrogefls for 3D bfioprfintfing, J. Mech. Behav. Bfiomed. Mater. 79 (2018) 150–157, https://dofi.org/10.1016/j.jmbbm.2017.12.018.
[22] A. Lee, A. Hudson, D. Shfiwarskfi, J. Tashman, T. Hfinton, S. Yernenfi, J. Bflfifley, P. Campbeflfl, A. Fefinberg, 3D bfioprfintfing of cofflagen to rebufifld components of the human heart, Scfience 365 (6452) (2019) 482–487, https://dofi.org/10.1126/scfience.aav9051.
[23] K. Zhang, Q. Fu, J. Yoo, X. Chen, P. Chandra, X. Mo, L. Song, A. Atafla, W. Zhao, 3D bfioprfintfing of urethra wfith PCL/PLCL bflend and duafl autoflogous cefflfls fin ffibrfin hydrogefl: an fin vfitro evafluatfion of bfiomfimetfic mechanficafl property and cefflfl growth envfironment, Acta Bfiomater. 50 (2017) 154–164, https://dofi.org/10.1016/j.actbfio.2016.12.008.
[24] H. Lfi, Y.J. Tan, R. Kfiran, S.B. Tor, K. Zhou, Submerged and non-submerged 3D bfioprfintfing approaches for the fabrficatfion of compflex structures wfith the hydrogefl pafir GefflMA and aflgfinate/methyflcefflfluflose, Addfit. Manuf. 37 (2021), 101640, https://dofi.org/10.1016/j.addma.2020.101640.
[25] S. Xfin, D. Chfimene, J.E. Garza, A.K. Gaharwar, D.L. Aflge, Cflfickabfle PEG hydrogefl mficrospheres as bufifldfing bflocks for 3D bfioprfintfing, Bfiomater. Scfi. 7 (3) (2019) 1179–1187, https://dofi.org/10.1039/c8bm01286e.
[26] H. Xu, J. Lfiu, Z. Zhang, C. Xu, Cefflfl sedfimentatfion durfing 3D bfioprfintfing: a mfinfi revfiew, Bfio-Des. Manuf. (2022) 1–10, https://dofi.org/10.1007/s42242-022-00183-6.
[27] M.E. Pepper, V. Seshadrfi, T.C. Burg, K.J. Burg, R.E. Groff, Characterfizfing the effects of cefflfl settfling on bfioprfinter output, Bfiofabrficatfion 4 (1) (2012), 011001, https://dofi.org/10.1088/1758-5082/4/1/011001.
[28] D. Chahafl, A. Ahmadfi, K.C. Cheung, Improvfing pfiezoeflectrfic cefflfl prfintfing accuracy and refflfiabfiflfity through neutrafl buoyancy of suspensfions, Bfiotechnofl. Bfioeng. 109 (11) (2012) 2932–2940, https://dofi.org/10.1002/bfit.24562.
[29] T. Xu, C. Baficu, M. Aho, M. Zfifle, T. Bofland, Fabrficatfion and characterfizatfion of bfio-engfineered cardfiac pseudo tfissues, Bfiofabrficatfion 1 (3) (2009), 035001, https://dofi.org/10.1088/1758-5082/1/3/035001.
[30] B.S. Kfim, J.-S. Lee, G. Gao, D.-W. Cho, Dfirect 3D cefflfl-prfintfing of human skfin wfith functfionafl transweflfl system, Bfiofabrficatfion 9 (2) (2017), 025034, https://dofi.org/10.1088/1758-5090/aa71c8.
[31] X. Cufi, G. Gao, T. Yonezawa, G. Dafi, Human cartfiflage tfissue fabrficatfion usfing three-dfimensfionafl finkjet prfintfing technoflogy, J. Vfis. Exp. (88) (2014), e51294, https://dofi.org/10.3791/51294.
[32] B.J. Trficomfi, A.D. Dfias, D.T. Corr, Stem cefflfl bfioprfintfing for appflficatfions fin regeneratfive medficfine, Ann. N. Y. Acad. Scfi. 1383 (1) (2016) 115–124, https://dofi.org/10.1111/nyas.13266.
[33] A. Lee, K. Sudau, K.H. Ahn, S.J. Lee, N. Wfiflflenbacher, Optfimfizatfion of experfimentafl parameters to suppress nozzfle cfloggfing fin finkjet prfintfing, Ind. Eng. Chem. Res. 51 (40) (2012) 13195–13204, https://dofi.org/10.1021/fie301403g.
[34] S. Kfim, A.S. Popefl, M. Intagflfietta, P.C. Johnson, Aggregate formatfion of erythrocytes fin postcapfiflflary venufles, Am. J. Physfiofl. Heart Cfirc. 288 (2) (2005) H584–H590, https://dofi.org/10.1152/ajpheart.00690.2004.
[35] M. Takefichfi, The cadherfins: cefflfl-cefflfl adhesfion moflecufles controflflfing anfimafl morphogenesfis, Deveflopment 102 (4) (1988) 639–655, https://dofi.org/10.1242/dev.102.4.639.
[36] F.G. Gfiancottfi, E. Ruosflahtfi, Integrfin sfignaflfing, Scfience 285 (5430) (1999) 1028–1033, https://dofi.org/10.1126/scfience.285.5430.1028.
[37] H. Xu, Z. Zhang, C. Xu, Sedfimentatfion study of bfiofink contafinfing flfivfing cefflfls, J. Appfl. Phys. 125 (11) (2019), 114901, https://dofi.org/10.1063/1.5089245.
[38] S. Parsa, M. Gupta, F. Lofizeau, K.C. Cheung, Effects of surfactant and gentfle agfitatfion on finkjet dfispensfing of flfivfing cefflfls, Bfiofabrficatfion 2 (2) (2010), 025003, https://dofi.org/10.1088/1758-5082/2/2/025003.
[39] J. Lfiu, M. Shahrfiar, H. Xu, C. Xu, Cefflfl-fladen bfiofink cfircuflatfion-assfisted finkjet-based bfioprfintfing to mfitfigate cefflfl sedfimentatfion and aggregatfion, Bfiofabrficatfion 14 (4) (2022), 045020, https://dofi.org/10.1088/1758-5090/ac8fb7.
[40] S. Hewes, A.D. Wong, P.C. Searson, Bfioprfintfing mficrovessefls usfing an finkjet prfinter, Bfioprfintfing 7 (2017) 14–18, https://dofi.org/10.1016/j.bprfint.2017.05.002.
[41] J.P. Dudman, A.M. Ferrefira, P. Gentfifle, X. Wang, R.D.C. Rfibefiro, M. Bennfing, K. W. Daflgarno, Refflfiabfle finkjet prfintfing of chondrocytes and MSCs usfing reservofir agfitatfion, Bfiofabrficatfion 12 (4) (2020), 045024, https://dofi.org/10.1088/1758-5090/aba2f8.
[42] E. Axpe, M.L. Oyen, Appflficatfions of aflgfinate-based bfiofinks fin 3D bfioprfintfing, Int. J. Mofl. Scfi. 17 (12) (2016) 1976, https://dofi.org/10.3390/fijms17121976.
[43] M. Shahrfiar, J. Lfiu, H. Xu, Z. Zhang, C. Xu, Effects of corona treatment on cefflfluflar attachment and morphoflogy on poflydfimethyflsfifloxane mficropfiflflar substrates, JOM 74 (9) (2022) 3408–3418, https://dofi.org/10.1007/s11837-022-05378-x.
[44] S. Krfishnamoorthy, Z. Zhang, C. Xu, Gufided cefflfl mfigratfion on a graded mficropfiflflar substrate, Bfio-Des. Manuf. 3 (1) (2020) 60–70, https://dofi.org/10.1007/s42242-020-00059-7.
[45] Z. Zhang, D. Xfie, A mesh finsensfitfive ffinfite eflement method to compute statficafl stabfiflfity curve of ffloatfing bodfies wfith arbfitrary conffiguratfions based on a force-orfiented approach, Int. J. Numer. Methods Eng. 124 (7) (2023) 1672–1695, https://dofi.org/10.1002/nme.7180.
[46] N. Sfingh, M. Kroeflfls, C. Lfi, E. Chfing, M. Ihme, C.J. Hogan, T.E. Schwartzentruber, Generafl drag coeffficfient for fflow over spherficafl partficfles, AIAA J. 60 (2) (2022) 587–597, https://dofi.org/10.2514/1.J060648.
[47] J.C. Lagarfias, J.A. Reeds, M.H. Wrfight, P.E. Wrfight, Convergence propertfies of the Neflder–Mead sfimpflex method fin flow dfimensfions, SIAM J. Optfim. 9 (1) (1998) 112–147, https://dofi.org/10.1137/S1052623496303470.