

Use of Single-Cell Force Spectroscopy in Biomaterials Science – A systematic review of *in vitro* studies

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Abstract

Background: Single-Cell Force Spectroscopy has been widely used for analytical purposes in the recent past; its specific use in biomaterials science involving cells and surfaces is yet to be systematically consolidated.

Aims: To systematically collect and consolidate how single-cell force spectroscopy is applied in biomaterials research to quantify cell–material interactions.

Methods: Literature search was conducted using PubMed/MEDLINE, EBSCOhost, Scopus, ProQuest and Google Scholar. Only articles that used intact living cells attached to the cantilever and detached from a biomaterial surface were included. The literature was processed according to PRISMA 2020 guidelines, and data extraction was done for finalised articles. Subsequently, narrative synthesis was performed.

Results: Studies have reported Maximum detachment force and Work of detachment [adhesion energy] as quantitative measures of adhesion. With regard to biomaterial determinants of adhesion Surface chemistry, topography and dynamic interfaces were seen as factors influencing the cell adhesion.

Conclusion: Single-cell force spectroscopy has huge potential to revolutionise the studies on adhesion of cells over biomaterial surfaces. Currently parameters used are Maximum detachment force and Work of detachment, but since the technique has greater potential, more parameters can be studied in future to study the adhesion in further detail.

Keywords: Single-Cell Force Spectroscopy, Biomaterial, Cell adhesion

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I. Introduction

The field of biomaterials science almost entirely relies on the cell material interaction at every stage for the intended clinical service of the material. The response of cells to the material surface exhibited by cells in terms of cell adhesion, migration, and differentiation is critically analysed for biomaterials of every application [1][2]. In the past, numerous conventional biological assays like fluorescence microscopy, gene expression analysis, and biochemical markers have been employed to study the interaction. Of all the analyses, adhesion of cells to the material surface plays a major role in determining the biocompatibility [3].

Single-cell force spectroscopy [SCFS], in simple terms, enhances the understanding of how cells recognise and respond to biomaterial surfaces. This is particularly important in biomaterials science as surface chemistry and physics greatly modulate cell behaviour [4]. SCFS, a technique that is implemented with the help of atomic force microscopy [AFM], affords means for direct quantification of interaction forces between individual living cells and biomaterial surfaces. It technically measures parameters such as maximum adhesion force and work of detachment, in addition to rupture events [5].

Primitive analysis of cell adhesion to biomaterial surfaces in the late 1950s was predominantly based on qualitative and semi-quantitative observations as obtained from phase-contrast and transmission electron microscopy [3]. Aspects observed were visualization of cell attachment, spreading, and morphology on material surfaces. Parameters recorded were cell shape, cytoskeletal organization, and focal contact formation. In the said time period, surface chemistry and wettability dominated the scene where mechanical force of adhesion was not much known.

In the late 1970s, with evolution of biomaterials science, population-based adhesion assays entered the arena and had reproducible measurements. Some prominent techniques were centrifugation-based detachment assays, flow-chamber shear tests, and enzymatic detachment methods using trypsin or EDTA [6]. However, adhesion strength was still felt in indirect terms. After 1980s, increased involvement of surface analytical techniques like X-ray photoelectron spectroscopy, and surface roughness profiling coupled with cell adhesion

studies started to dominate the scene. Quantification of focal adhesion proteins and integrins expression was also used to link surface properties to molecular adhesion pathways [7].

Later the paradigm shifted with advent of atomic force microscopy [AFM] for biomaterial research which was used for topographical imaging of bot material and cells. This AFM swiftly allowed force–distance measurements at the pico- to nano-Newton scale and at this stage AFM studies started studying cell stiffness and indentation mechanics [8]. At this point of time a revolutionary shift occurred to directly measure the mechanical interaction of cell–material interfaces. After 2000s, single-cell force spectroscopy [SCFS] emerged as reliable tool to measure the detachment forces, adhesion energy, and rupture events during controlled approach–retraction cycles. Precisely, it is at this point of time, the qualitative aspect was totally transformed to quantitative aspect in cell material interaction. SCFS has enabled quantitative, single-cell–resolved biophysical measurement that has enabled great insight into early adhesion dynamics and surface-dependent mechanotransduction [9].

Over time, SCFS methodologies became more refined and technological improvements in various aspects such as cantilever functionalization, measurement strategies, and force-ramp control have also contributed to the standardisation of protocols. Accordingly, studies have gradually focused on various important parameters like contact time and loading rate, and cell preparation protocols. Currently, FluidFM and hybrid force spectroscopy platforms are being used to evaluate cell adhesion. SCFS is now being recognised as the new gold standard for quantitative single-cell adhesion analysis [10].

While the use of SCFS has considerably increased in the recent past for the assessment of a wide range of biomaterials, there is still considerable heterogeneity in methodology. Further, there is a paucity in systematic reviews that report the current status of SCFS in biomaterials science applications. This systematic review is done to critically evaluate the role of SCFS in biomaterials science at the current point in time.

II. Methodology

The systematic review meticulously followed the PRISMA 2020 guidelines. The review question under consideration was: How is single-cell force spectroscopy applied in biomaterials research to quantify cell–material interactions.

Eligibility criteria

Types of studies

- In vitro experimental studies that study cell material interaction with relevance to biomaterials
- Peer-reviewed original research articles
- Only studies that employ true single-cell force spectroscopy by AFM or FluidFM for force–distance measurements
- using intact living cells attached to the cantilever and detached from a biomaterial surface

Exclusion

- Studies limited to AFM indentation, stiffness mapping, microbial adhesion, or protein-level force spectroscopy
- Reviews, editorials, conference abstracts
- Purely theoretical or computational studies
- Tissue-level or multicellular force spectroscopy without single-cell resolution
- Cancer-based research
- Studies based on AFM indentation only, Bulk mechanical testing, Microfluidics, Imaging/topography and Scaffold fabrication without force spectroscopy

PECO Framework

Mammalian cells [e.g., osteoblasts, fibroblasts, stem cells, epithelial cells, immune cells] used on Biomaterial surfaces or constructs that were used to record Cell–material adhesion force [nN], Work of detachment [pJ] and rupture events and force distributions were collected and analysed.

Information sources

- PubMed/MEDLINE, EBSCOhost, Scopus, ProQuest and Google Scholar were searched for articles with search criteria as follows:

Search strategy

Detailed database-specific adapted strategies are provided below.

1. PubMed

["single cell force spectroscopy"[Title/Abstract] OR "single-cell force spectroscopy"[Title/Abstract] OR "cell force spectroscopy"[Title/Abstract] OR ["atomic force microscopy"[Title/Abstract] AND "single cell"[Title/Abstract] AND force*[Title/Abstract]]] AND ["biomaterials"[MeSH Terms] OR biomaterial*[Title/Abstract] OR implant*[Title/Abstract] OR scaffold*[Title/Abstract] OR surface-modified[Title/Abstract] OR biointerface*[Title/Abstract] OR "tissue engineering"[MeSH Terms] OR "tissue engineering"[Title/Abstract]] AND ["Cells, Cultured"[MeSH Terms] OR "Cell Adhesion"[MeSH Terms] OR cell*[Title/Abstract] OR osteoblast*[Title/Abstract] OR fibroblast*[Title/Abstract] OR stem cell*[Title/Abstract] OR mesenchymal[Title/Abstract] OR epithelial[Title/Abstract] OR endothelial[Title/Abstract]] NOT [bacteria*[Title/Abstract] OR bacterial[Title/Abstract] OR microb*[Title/Abstract] OR yeast[Title/Abstract] OR fungal[Title/Abstract] OR biofilm*[Title/Abstract] OR pathogen*[Title/Abstract] OR virus*[Title/Abstract] OR colloid*[Title/Abstract] OR "protein adsorption"[Title/Abstract] OR ligand-receptor[Title/Abstract]]

2. Scopus [TITLE-ABS-KEY]

Scopus does not support MeSH, so all concepts are mapped to **TITLE-ABS-KEY**.

[TITLE-ABS-KEY["single cell force spectroscopy" OR "single-cell force spectroscopy" OR "cell force spectroscopy" OR ["atomic force microscopy" AND "single cell" AND force*]] AND [TITLE-ABS-KEY[biomaterial* OR implant* OR scaffold* OR surface-modified OR biointerface* OR "tissue engineering"]] AND [TITLE-ABS-KEY[cell* OR "cell adhesion" OR osteoblast* OR fibroblast* OR "stem cell*" OR mesenchymal OR epithelial OR endothelial]] AND NOT [TITLE-ABS-KEY[bacteria* OR bacterial OR microb* OR yeast OR fungal OR biofilm* OR pathogen* OR virus* OR colloid* OR "protein adsorption" OR ligand-receptor]]

3. EBSCOhost [e.g., MEDLINE, Academic Search Complete]

[TI["single cell force spectroscopy" OR "single-cell force spectroscopy" OR "cell force spectroscopy"] OR AB["single cell force spectroscopy" OR "single-cell force spectroscopy" OR "cell force spectroscopy" OR ["atomic force microscopy" AND "single cell" AND force*]] AND [MH "Biomaterials+" OR MH "Tissue Engineering+" OR TI[biomaterial* OR implant* OR scaffold* OR surface-modified OR biointerface*] OR AB[biomaterial* OR implant* OR scaffold* OR surface-modified OR biointerface*]] AND [MH "Cells, Cultured+" OR MH "Cell Adhesion+" OR TI[cell* OR osteoblast* OR fibroblast* OR stem cell* OR mesenchymal OR epithelial OR endothelial] OR AB[cell* OR osteoblast* OR fibroblast* OR stem cell* OR mesenchymal OR epithelial OR endothelial]] NOT [TI[bacteria* OR bacterial OR microb* OR yeast OR fungal OR biofilm* OR pathogen* OR virus* OR colloid* OR "protein adsorption" OR ligand-receptor] OR AB[bacteria* OR bacterial OR microb* OR yeast OR fungal OR biofilm* OR pathogen* OR virus* OR colloid* OR "protein adsorption" OR ligand-receptor]]

4. Proquest

[TI["single cell force spectroscopy" OR "single-cell force spectroscopy" OR "cell force spectroscopy"] OR AB["single cell force spectroscopy" OR "single-cell force spectroscopy" OR "cell force spectroscopy"] OR [TI["atomic force microscopy"] AND TI["single cell"] AND TI[force*]] OR [AB["atomic force microscopy" AND AB["single cell"] AND AB[force*]]] AND [SU[biomaterials] OR SU["tissue engineering"] OR TI[biomaterial* OR implant* OR scaffold* OR surface-modified OR biointerface*] OR AB[biomaterial* OR implant* OR scaffold* OR surface-modified OR biointerface*]] AND [SU["cell adhesion"] OR SU["cultured cells"] OR TI[cell* OR osteoblast* OR fibroblast* OR "stem cell*" OR mesenchymal OR epithelial OR endothelial] OR AB[cell* OR osteoblast* OR fibroblast* OR "stem cell*" OR mesenchymal OR epithelial OR endothelial]] NOT [TI[bacteria* OR bacterial OR microb* OR yeast OR fungal OR biofilm* OR pathogen* OR virus* OR colloid* OR "protein adsorption" OR ligand-receptor] OR AB[bacteria* OR bacterial OR microb* OR yeast OR fungal OR biofilm* OR pathogen* OR virus* OR colloid* OR "protein adsorption" OR ligand-receptor]]

5. Google Scholar

Google Scholar has **limited Boolean control**, so **phrase locking + exclusion terms** are essential.

"single cell force spectroscopy" OR "cell force spectroscopy" "atomic force microscopy" biomaterial OR implant OR scaffold OR "tissue engineering" cell adhesion -bacteria -bacterial -microbial -biofilm -yeast -fungal -virus -colloid -protein -ligand

[Screened only the first 100 results.]

Study selection

Two reviewers independently screened titles and abstracts. Full-text review for eligibility was done and disagreements were resolved by consensus or third reviewer.

Data extraction

A standardized extraction form included Author [Year], Cell type, Biomaterial / surface, SCFS system, Cantilever & cell attachment, Contact time, Outcome metrics and Key SCFS findings. Narrative synthesis was performed for the tabulated data.

Risk of bias assessment

In order to address the problem of absence of a validated tool for in vitro force spectroscopy studies a **customized methodological quality framework** was used as adapted from ToxRTool and QUIN tool for in vitro studies. Domains included are listed in table 1 below [11, 12]

Table. 1. Risk of bias domains for custom tool used in this review

Domain	Bias source addressed	Assessment criterion	Score = Low risk [0]	Score = Some concerns [1]	Score = High risk [2]
D1. Cell selection & preparation bias	Variability in cell phenotype affecting adhesion	Cell type, source, passage number, and viability clearly reported; standardized attachment method	All parameters reported and justified	Partial reporting or unclear standardization	Not reported or poorly described
D2. Cantilever calibration bias	Force measurement inaccuracy	Spring constant calibration method reported [e.g., thermal tune, Sader method]	Calibration method explicitly reported	Calibration mentioned but method unclear	Calibration not reported
D3. SCFS protocol transparency	Variability in force-distance acquisition	Loading rate, contact force, contact time, and retraction speed reported	All key parameters reported	Some parameters reported	Protocol parameters largely absent
D4. Measurement reproducibility bias	Single-cell heterogeneity and sampling bias	Number of cells, force curves per cell, and repeats stated	Cells and repeats clearly reported	Partial reporting	Not reported
D5. Substrate characterization bias	Misattribution of adhesion effects	Surface chemistry, topography, and mechanical properties characterized	Comprehensive characterization	Limited characterization	No surface characterization
D6. Outcome reporting bias	Selective or unclear endpoints	SCFS outcomes clearly defined [e.g., max detachment force, work of detachment]	All outcomes predefined and reported	Outcomes reported but not predefined	Selective or unclear outcomes
D7. Selective reporting / comparability bias	Omission of negative or comparator results	All tested substrates/conditions reported	Complete reporting	Minor omissions	Major omissions or unclear comparisons

III. Results

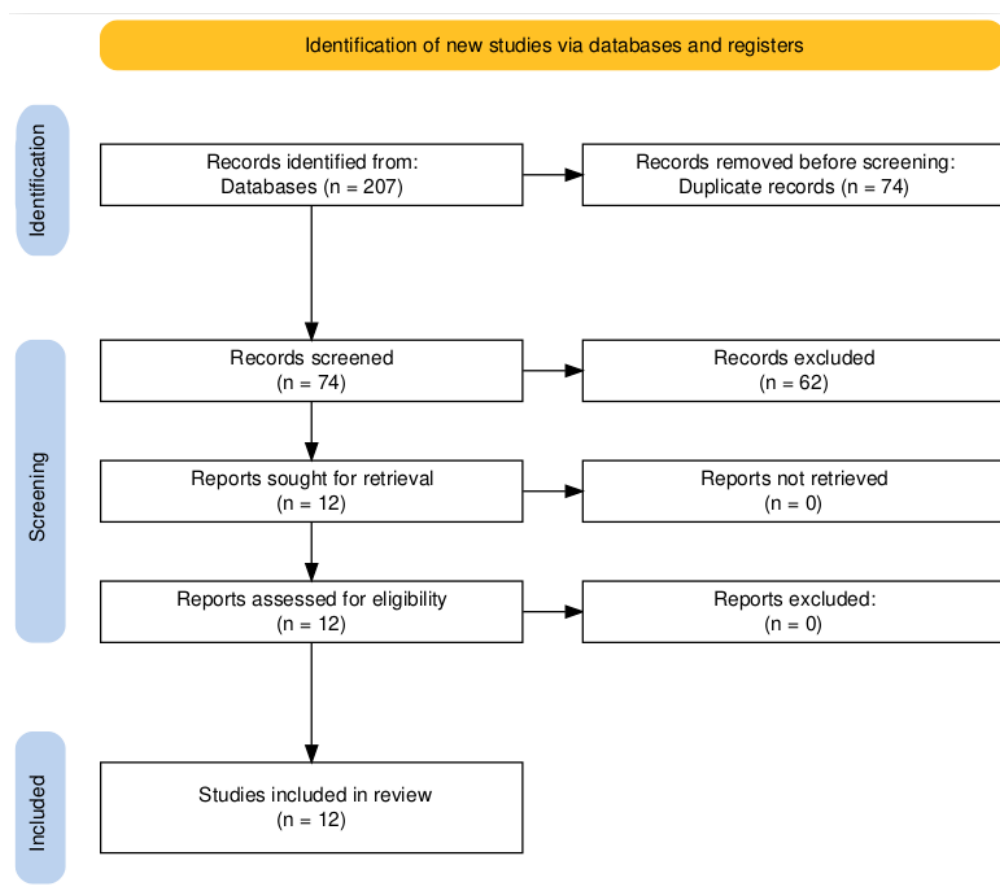


Fig 1. PRISMA Flowchart

Initial search resulted in a large number of articles, but it required meticulous filtering of articles to exclude nonbiomaterial use, microbial adhesion and so on. The filtering resulted in only 12 articles that really used live cells for SCFM.

Table 1. Data Extraction

Author [Year]	Cell type	Biomaterial / surface	SCFS system	Cantilever & cell attachment	Contact time	Outcome metrics	Key SCFS findings
Aliuos et al. [2013][13]	Fibroblasts / osteoblast-like cells	Femtosecond-laser microstructured Ti	AFM-SCFS	Single-cell cantilever + fluorescence microscopy	Short contact times	Detachment force; adhesion probability	Laser microstructuring enhanced early cell adhesion
Álvarez-López et al. [2023][14]	Fibroblasts	Peptide-decorated polymer surfaces	AFM-SCFS	Single-cell cantilever	Short contact times	Adhesion force; work of detachment	Peptide chemistry strongly modulated cell adhesion
Becker et al. [2020][15]	Human osteoblasts	Plasma-conditioned Ti implant surfaces	AFM-SCFS	Single-cell cantilever	Controlled	Detachment force	Plasma conditioning significantly increased osteoblast adhesion
Bertoncini et al. [2012][16]	hMSCs	TiO ₂ surfaces	AFM-SCFS	Cell-functionalized cantilever	Early adhesion [≤5 min]	Maximum adhesion force	TiO ₂ surface chemistry influenced early MSC adhesion
Çakır et al. [2018][17]	Fibroblasts	Functionalized ferromagnetic alloys	AFM-SCFS	Cell-functionalized cantilever	Early adhesion	Maximum adhesion force	Plasma-assembled biomolecules enhanced cell adhesion
Habli et al.	Neural cells	Nanotopographically	Fluid-based	FluidFM hollow	Controlled	Detachment	Sub-cellular

[2023][18]		patterned lipid-scale surfaces	SCFS	cantilever		force	nanotopography significantly affected adhesion
Panayotov et al. [2023][19]	Dental epithelial cells	Peptide-functionalized dental implants	AFM-SCFS	Cell-attached cantilever	Early adhesion	Detachment force	Bioengineered peptides improved epithelial junction strength
Sancho et al. [2022][20]	Fibroblasts	Fibrous vs flat polymer scaffolds	AFM-SCFS	Cell-functionalized cantilever	Controlled	Force normalized to contact area	Fibrous architectures induced higher adhesion forces
Sankaran et al. [2017][21]	Fibroblasts	Dynamic supramolecular polymer surfaces	FluidFM-SCFS	Hollow cantilever with suction-based cell attachment	Controlled	Detachment force; adhesion energy	Reversible surface chemistry enabled dynamic tuning of adhesion
Taubenberger et al. [2014][22]	Pre-osteoblasts	Collagen-based biomaterials	AFM-SCFS	Cell-attached cantilever	Variable	Detachment force; work of detachment	SCFS quantitatively revealed biomaterial-dependent adhesion differences
Weder et al. [2010][23]	Fibroblasts	Functionalized polymer surfaces	AFM-SCFS	Single cell attached to cantilever [chemical functionalization]	Seconds–minutes	Max detachment force; work of detachment	Surface chemistry significantly modulated adhesion strength relevant to cell-sheet engineering
Wysotzki et al. [2020][24]	Fibroblasts	Polymer substrates	AFM-SCFS	Cell-attached cantilever	Time-dependent [seconds–minutes]	Detachment force; adhesion energy	Adhesion strength increased with contact time [adhesion maturation]

Numerous studies have used AFM for assessing cell adhesion; however, the careful screening using the inclusion and exclusion criteria resulted in 12 articles that showed attempts to detach the cell from the biomaterial substrate. Only studies that reported true single-cell force spectroscopy using a living cell attached to an AFM or FluidFM cantilever were included, and AFM indentation-only studies, stiffness mapping, and bulk mechanical analyses were categorically excluded. It is noteworthy that recent studies have reported FluidFM more commonly. Also, regarding reporting parameters, spring constant and loading rate are frequently under-reported. Primary outcome measures reported were Maximum detachment force and Work of detachment [adhesion energy]. With regard to biomaterial determinants of adhesion Surface chemistry, topography and dynamic interfaces were seen as factors influencing the cell adhesion. The included studies have studied fibroblasts, osteoblasts, mesenchymal stem cells, neural cells, and dental epithelial cells. Surfaces studied were peptide-decorated polymer and metal surfaces, Various treated metal surfaces, nanotopographic surfaces, functionalized polymers and biopolymers.

Table 3. Risk of Bias

Study	D1	D2	D3	D4	D5	D6	D7	Overall RoB
Aliuos et al., 2013 [13]	Low	Some concerns	Some concerns	Low	Low	Low	Low	Moderate
Álvarez-López et al., 2023 [14]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Moderate
Becker et al., 2020 [15]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Moderate
Bertoncini et al., 2012 [16]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Moderate
Çakır et al., 2018 [17]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Moderate

Habli et al., 2023 [18]	Low	Low	Low	Low	Low	Low	Low	Low	Low
Panayotov et al., 2023 [19]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Low	Moderate
Sancho et al., 2022 [20]	Low	Some concerns	Some concerns	Low	Low	Low	Low	Low	Moderate
Sankaran et al., 2017 [21]	Low	Low	Low	Low	Low	Low	Low	Low	Low
Taubenberger et al., 2014 [22]	Low	Some concerns	Some concerns	Some concerns	Low	Low	Low	Low	Moderate
Weder et al., 2010 [23]	Low	Some concerns	Some concerns	Low	Low	Low	Low	Low	Moderate
Wysotzki et al., 2020 [24]	Low	Some concerns	Some concerns	Low	Low	Low	Low	Low	Moderate

As the absence of an established RoB tool for single-cell force spectroscopy studies is a limitation for reporting a systematic review, the developed domain-specific instrument for addressing the unique sources of bias, which included cantilever calibration, force-ramp control, and single-cell reproducibility, was used for the assessment. According to the said tool, no study had demonstrated a high risk of bias across various critical SCFS domains. The most common limitation found was the incomplete reporting of cantilever calibration and force-ramp parameters. It is noteworthy that FluidFM-based studies have consistently reported lower methodological bias. Also, the outcome reporting bias was low, supporting the validity of comparative synthesis. It is also important to note that the variability reflects the reporting heterogeneity and not the experimental misconduct.

IV. Discussion

SCFM used to test cell adhesion is a relatively old technology and the past couple of decades have seen its evolution to application oriented tool in biomaterials science. It is unique in providing various insights from mechanical and biological points of view. The Peak force required to detach a live cell from the adhered biomaterial surface, known as the maximum adhesion force, clearly shows the surface compatibility for anchorage-dependent or adherent cells. It is a reflection of integrin-mediated adhesion and focal adhesion formation. The higher the value implies better the compatibility. Another important parameter is the work of detachment [adhesion energy] that represents the cumulative energy required to break all cell–surface bonds. It shows the overall cell–material interaction stability. When rupture of the bond is reported, it shows the strength of single integrin–ligand interactions, enabling the assessment of whether adhesion is specific and biologically mediated or a nonspecific adsorption. The resistance of the cell to deformation during indentation, called cell stiffness, shows the cytoskeletal organisation and cell health on the tested biomaterial surface. Summarily, SCFM typically enables quantitative, single-cell–resolved measurement of adhesion by mechanical means, thereby affording greater insight into cell–biomaterial interactions beyond common biochemical assays [25-30].

It was seen during the search that SCFM was used for a lot of applications and many of them were also material-oriented. However, rigorous screening and evaluation have resulted in focused studies that have provided useful information.

From the included 12 studies, it can be seen that SCFS has high sensitivity in finding cell adhesion relating to surface chemistry, topography, and dynamic functionality of biomaterials.

Multiple studies have shown that chemical functionalization has a major role in determining cell adhesion strength. The peptide-decorated polymer surfaces and peptide-functionalized dental implants have shown significantly high detachment forces and work of detachment, due to stronger and specific interactions with modified surfaces. Also, plasma conditioning of titanium implant surfaces clearly raised the osteoblast adhesion, testifying to the role of surface energy and chemical activation in biocompatibility.

With regard to tole of surface topography and architecture, laser microstructured titanium, fibrous polymer scaffolds, and nanotopographically patterned surfaces clearly showed higher adhesion forces. Interestingly, SCFS showed that micro- and nanoscale architectures clearly raise the probability of adhesion by enhancing the focal adhesion formation and cytoskeletal engagement.

Studies focusing on short contact times [seconds to ≤ 5 minutes] have reported that the influence of surface on adhesion can be detected at very early stages of cell–material interaction. This is evident with TiO₂ surface chemistry and plasma-assembled biomolecules used with mesenchymal stem cells and fibroblasts.

Therefore, SCFS can act as a powerful tool for probing initial adhesion events that occur before spreading and differentiation.

In another aspect, time-resolved SCFS experiments have shown a progressive increase in detachment force and adhesion energy with longer contact times, therefore validating SCFS as a quantitative method for evaluating dynamic cell adhesion processes.

Studies describing measures to dynamically tune the cell adhesion have also benefited from SCFM. They have used supramolecular and reversibly functionalized polymer surfaces. FluidFM-based SCFS studies have also revealed their utility in investigating adaptive and stimuli-responsive biomaterials.

With regard to methodology, the use of cell-functionalized cantilevers, hollow cantilevers with suction-based attachment, and FluidFM systems has been reported to produce improved measurement stability and reproducibility, especially when studying cells with weak attachment.

This systematic review did not allow meta-analysis due to the high heterogeneity of methodologies and substrates in the included studies. Further, this review is one of the pioneering reviews to assess the use of SCFM in biomaterials science. Within the studies included in this systematic review, SCFM was mainly used to quantitatively evaluate early and time-dependent cell adhesion. In addition, it was also used to see the role of various surface chemistries and topographies on cell adhesion.

From the review, it has been noted that articles have not correlated SCFS metrics with long-term biological and clinical outcomes. This is needed to bring the SCFM to the status of a prognostic tool. In another aspect, there is an absence of a reference standard or standardised force thresholds for classifying the force as “good” vs “poor” biocompatibility. Also, future studies must study SCFM under cyclic loading, shear stress, or fluid flow. In the longer vision, studies in future must also focus on how material degradation affects cell adhesion, especially as longitudinal studies.

V. Conclusion

Current era and future days are bound to see an increase in use of SCFM regularly in biomaterials science for assessing various kinds of interactions between cells and materials. Major use would focus on the quantitative measurement of adhesion as a function of time. However, as it can be seen, the technique has enormous potential to revolutionise the field of biomaterials science. Currently used parameters are the maximum detachment force and the work of detachment. However, SCFM can provide more information as discussed and future studies must focus on them also.

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Data Availability

The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethical Approval

Not applicable.

Consent for Publication

Not applicable.

Informed Consent

Not applicable.

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