Development of polymer systems suitable for processing via advanced technologies of 3D printing and electrospinning

Ing. Lenka Vítková, Ph.D.

Doctoral Thesis Summary

Tomas Bata University in Zlín

Tomas Bata University in Zlín
Faculty of Technology

Doctoral Thesis Summary

Development of polymer systems suitable for processing via advanced technologies of 3D printing and electrospinning

Vývoj polymerních systémů vhodných pro zpracování pomocí pokročilých technologií 3D tisku a elektrostatického zvlákňování

Author: **Ing. Lenka Vítková, Ph.D.**

Degree programme: P2808/ Chemistry and Technology of Materials

Degree course: 2808V006/ Technology of Macromolecular Compounds

Supervisor: prof. Mgr. Aleš Mráček, Ph.D.

External examiners: doc. Dr. Ing. Vladimír Pavlínek
prof. RNDr. Petr Ponížil, Ph.D.

Zlín, August 2023

Published by **Tomas Bata University in Zlín** in edition **Doctoral Thesis Summary** in 2023.

Klíčová slova: *aditivní výroba, biopolymery, hyaluronan, hydrogely, 3D biotisk, elektrostatické zvlákňování*

Key words: *additive manufacturing, biopolymers, hyaluronan, hydrogels, 3D bioprinting, electrospinning*

The full version of doctoral thesis is available in the Library of TBU in Zlín.

ISBN 978-80-7678-182-5

ABSTRAKT

Pokročilé technologie aditivní výroby umožňují zpracování polymerů do komplikovaných struktur s různými geometriemi, porozitou a mechanickými vlastnostmi. S využitím elektrického pole lze dosáhnout vetších detailů. Aditivní charakter zpracování v kombinaci s multimateriálovou výrobou umožňuje optimalizovat geometrii kompozitů a vysokou přesností na několika úrovních. Tento způsob výroby se využívá v mnoha oborech, například v biomedicíně a tkáňovém inženýrství pro výrobu tkáňových nosičů s chemickými, mechanickými a strukturními vlastnostmi upravenými na míru dané tkáni, čímž je umožněna úspěšná kultivace buněk. Cílem této práce je prozkoumat možnosti zpracování přírodních polymerů pomocí pokročilých technologií 3D tisku a elektrostatického zvlákňování. K tomuto účelu jsou vybrány polymery pro výrobu hydrogelových matric jako inkoustů vhodných pro mikroextruzní 3D tisk. Dále jsou roztoky těchto polymerů zvlákňovány v elektrickém poli. Tyto procesy jsou optimalizovány na základě vlivu různých parametrů, aby bylo dosaženo požadovaných výsledků. Při kombinaci obou technologií lze výsledky této práce využít pro vytvoření tkáňových nosičů obsahující nanostruktury, které poskytnou věrný analog přirozeného prostředí pro kultivaci buněk.

ABSTRACT

Advanced additive manufacturing technologies provide means to precisely fabricate elaborate structures with various geometries, porosity and mechanical performance. Additionally, electric field assisted polymer processing can be used to fabricate fine structures with nanotopographical features. The additive manufacturing processes - specifically 3D printing and electrospinning, which are the central focus of the current thesis - are increasingly popular in medical fields, such as tissue engineering, due to their versatility. They present a powerful tool to optimize the scaffolds from chemical, mechanical and structural points of view to mimic specific tissue types for cell cultivation. This thesis examines the possibilities of processing of natural polymers by the advanced technologies of 3D printing and electrospinning. To this end, the selected polymers are used as hydrogel matrices fabrication for preparation of inks suitable for microextrusion 3D printing. Alternatively, polymer solutions

are spun into nanofibres in an electric field. The specific criteria of each process are followed to allow tuning of the process. Ultimately, the results of both technologies could be combined to achieve fabrication of scaffold containing nanostructures, and thus provide precise tissue analogue for cell cultivation.

LIST OF PAPERS

PAPER I

L. Musilová, E. Achbergerová, **L. Vítková**, R. Kolařík, M. Martínková, A. Minařík, A. Mráček*, P. Humpolíček and J. Pecha. Cross-Linked Gelatine by Modified Dextran as a Potential Bioink Prepared by a Simple and Non-Toxic Process. *Polymers*. 2022, 14(3). doi:10.3390/polym14030391

PAPER II

L. Vítková, L. Musilová*, E. Achbergerová, R. Kolařík, M. Mrlík, K. Korpasová, L. Mahelová, Z. Capáková and A. Mráček*. Formulation of Magneto-Responsive Hydrogels from Dually Cross-Linked Polysaccharides: Synthesis, Tuning and Evaluation of Rheological Properties. *International Journal of Molecular Sciences*. 2022, 23(17). doi:10.3390/ijms23179633

PAPER III

L. Vítková, I. Smolková, N. Kazantseva, L. Musilová, P. Smolka*, K. Valášková, K. Kocourková, M. Humeník, A. Minařík, P. Humpolíček and A. Mráček. Magneto-responsive hyaluronan hydrogel for hyperthermia and bioprinting: magnetic, rheological properties and biocompatibility. *APL Bioengineering*. Under review.

PAPER IV

K. Kopecká, **L. Vítková***, Z. Kroneková, L. Musilová, P. Smolka, F. Mikulka, K. Melánová, P. Knotek, M. Humeník, A. Minařík and A. Mráček*. Synthesis and exfoliation of calcium phosphonates for tailoring rheological properties of sodium alginate solutions: A path towards polysaccharide based bioink. *Biomacromolecules*. Accepted for publication. doi:10.1021/acs.biomac.3c00081

PAPER V

L. Vítková, L. Musilová, E. Achbergerová, A. Minařík, P. Smolka, E. Wrzecionko and A. Mráček*. Electrospinning of Hyaluronan Using Polymer Coelectrospinning and Intermediate Solvent. *Polymers*. 2019, 11(9).doi:10.3390/polym11091517

TABLE OF CONTENTS

1 INTRODUCTION

Advanced manufacturing is an overarching terms for innovative processes and techniques utilizing technologies such as additive manufacturing, nanotechnology, or advanced materials manufacturing [1]. These technologies are designed to reduce waste and thus enable more efficient product manufacturing. Contrary to subtractive manufacturing, additive manufacturing employs a bottom-up approach that can revolutionize the creation of complex shapes and structures.

3D printing and electrospinning employ the bottom-up approach to create 3D structures from various materials. 3D printing uses a computer-controlled printer to deposit layers of a material to construct a 3D structure, producing structures with a resolution of thousands to hundreds of micrometers [2, 3]. Electrospinning utilizes an electric field to produce a fine fibres of hundreds to tens of nanometers in thickness, although it typically leads to random nanofibrous meshes and thus is considered less precise than 3D printing [4].

Both 3D printing and electrospinning are used in tissue engineering, providing scaffolds for cell culture and tissue repair [5]. 3D printing provides control over macroscopic geometry, while electrospinning is faster and produces fibres with smaller diameters. The materials research has provided a large number of compositions utilizable in both technologies respectively. Each technology contributes distinct qualities to achieve cell guidance through the presence of signal functional groups and topographical cues which influence cell behavior [6, 7]. Thus, combining 3D printing and electrospinning can be seen as a biomimetic approach to creating structures suitable for tissue engineering or pharmaceutical applications.

2 STATE OF THE ART

Processing of polymer systems using advanced technologies offers remarkable opportunities in various areas of research, including medical fields such as drug delivery, wound healing, tissue engineering, and cell scaffold fabrication [8, 9, 10, 11, 12]. Polymer systems intended for medical applications must adhere to specific re-

quirements involving the *in vivo* interactions. Particularly in tissue engineering, the polymer systems should imitate native tissue properties for optimal cell adhesion and proliferation [13].

The requirements include for example providing support and allowing communication among cells [14], which is facilitated by the extracellular matrix (ECM) in native tissue. These requirements are fulfilled by high water content and porosity, making hydrogel materials popular [6]. Nanoscale structures play a significant role in cell cultivation, influencing cell division, morphology, and tissue formation [15, 16, 17, 7, 18]. Mechanical properties of a tissue vary according to their function. For example, Young‘s modulus can range from tens of GPa for cancellous bone [19] through units of MPa found for muscles [20] to hundreds of kPa in case of adipose tissue types [21]. Some tissues also display anisotropy, providing a different response based on the direction of impulse. Engineering of such tissue types benefits from introducing a gradient of the desired quality to the scaffold structure [22].

An emergent approach to cell scaffold fabrication is the use of smart materials, which respond instantly and reversibly to external stimuli, including temperature, electric or magnetic field, light, or humidity [23]. Smart materials have been used to induce chondrogenesis in cells [24], stimulate fibroblasts proliferation [25] or create magneto-responsive cell scaffolds [26, 27] or thermally controlled drug delivery systems [28]. The combination of smart materials and advanced manufacturing offers the potential to achieve 4D printing - 3D printing of structures that change over time [29].

Cell cultivation on artificial scaffolds depends on surface properties, which influence cell attachment, migration, proliferation, and differentiation [30, 31, 32, 33, 34]. Additionally, 3D cell culture has proven more effective than 2D in creating viable tissue analogues, making it a bridge between cell culture and living tissue [14, 35]. The essence of 3D cell cultivation is in the cell-ECM and cell-cell interactions [36]. It can be achieved through bioinstructive cues in 3D cell culture guiding cell growth, proliferation, and differentiation [37].

In a recent study, Fornetti et al., 2023 observed the alignment provided by 3D printing also leads to better orientation of muscle cells [38]. Aligned electrospun nanofi-

bres, on the other hand, have also shown the capacity to guide cell proliferation and differentiation in the reconstruction of myocytes and neural cells [39, 40, 41, 7]. Furthermore, the use of nanofibres as fillers for hydrogel matrices has been shown to induce cell alignment [42]. It is apparent that combining 3D printing and electrospinning technologies is a promising approach for fabricating cell-instructive scaffolds for tissue engineering applications [5, 43, 44].

3 AIMS OF THE DOCTORAL THESIS

The main focus of the doctoral thesis is the development of natural polymer based systems suitable for processing *via* 3D printing and electrospinning technologies in order to obtain constructs distinctly structured on macro-, micro- and nanolevel. This is achieved through thorough examination and fine tuning of key parameters for the respective techniques. Furthermore, the possibility to utilize composite materials and enhance the polymer matrix performance will be studied. The research objective is directed towards fabrication of scaffolds for cell cultivation. Therefore, it is necessary to be mindful of biocompatibility in each step of the process.

4 THEORETICAL BACKGROUND

4.1 Polymers and polymer systems

Polymers present a group of materials consisting of macromolecules (several millions grams per mol), which are built from repeating units conceptually derived from low-molecular-mass molecules. Polymers can be divided into several groups based on their origin (natural or synthetic), primary structure (homopolymers or copolymers, which can be further divided into subgroups - statistical, alternating, block or graft copolymers), or secondary structure (linear, branched or cross-linked) [45]. Structural characteristics of a polymer chain, such as M_w, polydispersity, linearity etc. directly influence polymers mechanical [46, 47], rheological [48], or thermal properties [49].

Polymer systems comprise mixtures of polymers with other substances - solvents, fillers (either passive or active), cross-linking agents, other polymers etc. [50]. These additives influences the behaviour of the polymer chains through physical or chemical interaction. The variety of possible effects leads to almost infinite range of possible polymer systems. For the purposes of the thesis, only specific groups of polymer systems essential to the research will be described.

4.1.1 Polymer based scaffolds in tissue engineering

Tissue engineering involves three main elements: a scaffold, cells, and signaling pathways [51]. The scaffold provides a framework for cell attachment and mechanical, thus mimicking the functions of the ECM — a composite of proteins and glycosaminoglycans that plays a vital role in cellular adhesion and growth [1].

A scaffold's core feature is its high porosity, enabling cell growth within the pores and facilitating fluid flow. As such, hydrogels are extremely promising candidates in scaffold fabrication and ECM environment simulation, as the porosity and high water content are inherently present in their structure [6]. Furthermore, their relative ease of processing allows precise control over the shape of the scaffold [1].

Materials for tissue engineering must meet certain criteria, including biodegradability, biocompatibility and non-toxicity. Additionally, the presence of biologically active chemical cues, hydrophilicity, and overall chemical composition is also important [52]. Hydrogel matrices for scaffold fabrication can be in general obtained from both natural and synthetic polymers. While synthetic polymers offer high reproducibility, they lack cellular adhesive sites and present the risk of cytotoxicity [53]. Hence, their use for cell growth requires functionalization with signaling molecules [54]. Natural polymers, on the other hand, are non-toxic and include bioinstructive chemical cues in their structure [53], but they also lack predictability and thermal stability, making the complex shape processing challenging [52].

4.1.2 Polymer composites in tissue engineering

Single-component hydrogels properties are often insufficient for tissue engineering applications [55]. Thus, composite materials, which combine multiple materials in synergy for enhanced properties or additional functionality, are being developed. Precise structuring of composites introduces anisotropy to the material [56]. Certain types of composite filler provide platforms for stimuli-responsiveness and on-demand modulation, forming smart materials [57].

There are some significant types of fillers with distinct effect on the matrix behaviour, including fibres introducing directional anisotropy and reinforcement [58, 42]. Another form of composite fillers is the particles, or significantly nanoparticles (NPs). The growing popularity of nanosized fillers is due to the enhanced interfacial effects resulting from their increased specific surface [59]. Carbon-based NPs or bioceramics are among the most common nanofillers, providing conductivity or mechanical reinforcement [60]. Surface charged disc-like nanoclays, such as Laponite®, can provide intrinsic structural support to the polymer matrix, enhancing extrudability and recovery after shear. Thus, these fillers can serve as rheological modifiers for 3D printable hydrogels [61].

Certain kinds of nanoparticulate filler can also facilitate stimuli-responsiveness, thus providing smart hydrogels. As an example, magnetic particles can trigger certain behaviour upon application of a magnetic field. Commonly, the response is mechanical, as the presence of an external magnetic field induces ordering of the filler particles along the magnetic field, leading to increase of mechanical performance, also denoted as magneto-rheological efect (MRE) [62]. Biocompatible magneto-rheological hydrogels can be used e.g. as embolization agents [63], or to impose dynamic mechano-modulation on cells [64].

In a different application of magnetic particles, magneto-thermal responsiveness can be acheived. The principle of magneto-thermally responsive hydrogels is based on rapid periodical changes of magnetic moment direction induced by an alternating magnetic field, which leads to generation of heat. The heating is provided by hysteresis loss [65], Brown relaxation referring to the physical change of particles orientation [66], and Néel relaxation which describes the change of magnetic mo-

ment within the particle [67]. The particle size determines the dominant heating mechanism of a magneto-thermally responsive hydrogel. The produced heat can be used for various medical applications, including hyperthermia cancer treatment [67], thermal neurostimulation [68], or targeted drug delivery in combination with thermoresponsive polymer networks [69].

4.2 3D printing

3D printing as a layer-by-layer fabrication offers a great versatility in design of the product. Naturally, the technology provides great opportunities also in terms of pharmaceutical research such as temporally programmed drug release and targeted drug delivery [70]. Furthermore, its possibilities in medical use, especially wound treatment [71] and tissue engineering [72] are abundant.

3D printing in medical applications and especially bioprinting - i.e. printing of cell-laden biomaterials - draws attention of researchers, as it allows preparing complex structures as well as controlled distribution of cells [73]. This ability could diminish the shortage of transplantable organs supply [51], as well as allow cruelty-free pharmaceutical research [74, 75]. There are three basic options suitable for 3D printing of hydrogels: inkjet printing, extrusion-based printing and laser assisted printing [76].

Inkjet printing technology places the ink material dropwise on the predefined location using for example piezoelectric pulses [77], or thermal generation of vapour to pressurize the printhead [78]. In this case, high surface tension is crucial for high printing precision, as it increases the materials tendency to form droplets [79]. This method required low viscosity inks, thus the stability of the constructs needs to be ensured by *in situ* cross-linking [80].

In contrast to inkjet printing, extrusion printing technology generally produces strands of materials, which is placed on the substrate, which leads to lower resolution [81]. On the other hand, the variety of driving systems in this method (pneumatic, piston or screw) extends the range of usable inks to highly viscous materials, as long as they are shear thinning to ensure extrudability [80].

The basic principle of laser assisted printing can be likened to inkjet printing, as it also uses placing of small droplets of material to desired position. Conversely to the above listed techniques, laser assisted printing does not use nozzle based printheads, but the printing is realized through a plate composed of donor ribbon (i.e. the printing material), and absorbing layer. The absorbing layer is locally evaporated by a focused laser beam, creating a high-pressure bubble which forces a small droplet of printing material out of the donor ribbon [82]. The high resolution printing is only achieved for materials with rapid gelation, which ensures low spreading [83].

Table 4.1 shows that while the best results in terms of resolution and cell viability can be achieved by laser assisted bioprinting, the high costs prevent its use to expand. Additionally, like inkjet printing, it is unsuitable for high viscosity materials. As per Table 4.1, the generally highly viscous hydrogel can only be printed by extrusion based technologies.

Printing technology	**Advantages**	**Disadvantages**	**Ink material**
Inkjet	High resolution $>$ 85% cell viability	Low material viscosities (3.5-12 mPa·s) Poor vertical structure Low cell density	Alginate PEGDMA Collagen
Extrusion	Wide range of viscosities (30 mPa·s - 10^4 Pa·s) High cell density Good vertical structure	Moderate resolution 40%-80% cell viability	Alginate GelMA Collagen
Laser assisted	High resolution $>$ 95% cell viability Good vertical structure Fair cell density	Low viscosity materials (1-300 mPa·s) High cost	Collagen Matrigel

Tab. 4.1 Comparison of basic 3D printing technologies for tissue engineering [73]

4.2.1 Hydrogels for bioprinting

Polymeric hydrogels are one of the most useful materials for constructing 3D printed porous scaffolds due to their ECM-like qualities and ability to modulate cell be-

haviour, while retaining acceptable shape fidelity during printing process [84, 85]. Both synthetic and natural polymers are utilized, leveraging mechanical and chemical robustness of synthetic polymers, and bioadhesiveness of natural polymers[86].

Hydrogels based bioinks are constrained by three essential parameters: printability, cross-linkability, and biocompatibility. Printability is described as the capacity to accurately control the positioning of the bioink, and it is dictated by the bioink's rheological characteristics [87]. Cross-linkability ensures scaffold stability during cell cultivation [73] through inducing the formation of a 3D polymer network. Common cross-linking strategies include photo- [88, 89], chemical [3], or thermal [90] cross-linking. Biocompatibility, which extends beyond immunogenicity to encompass adhesiveness, proliferation support, and even stem cell differentiation, represents the third key characteristic [87, 91, 92, 93, 35].

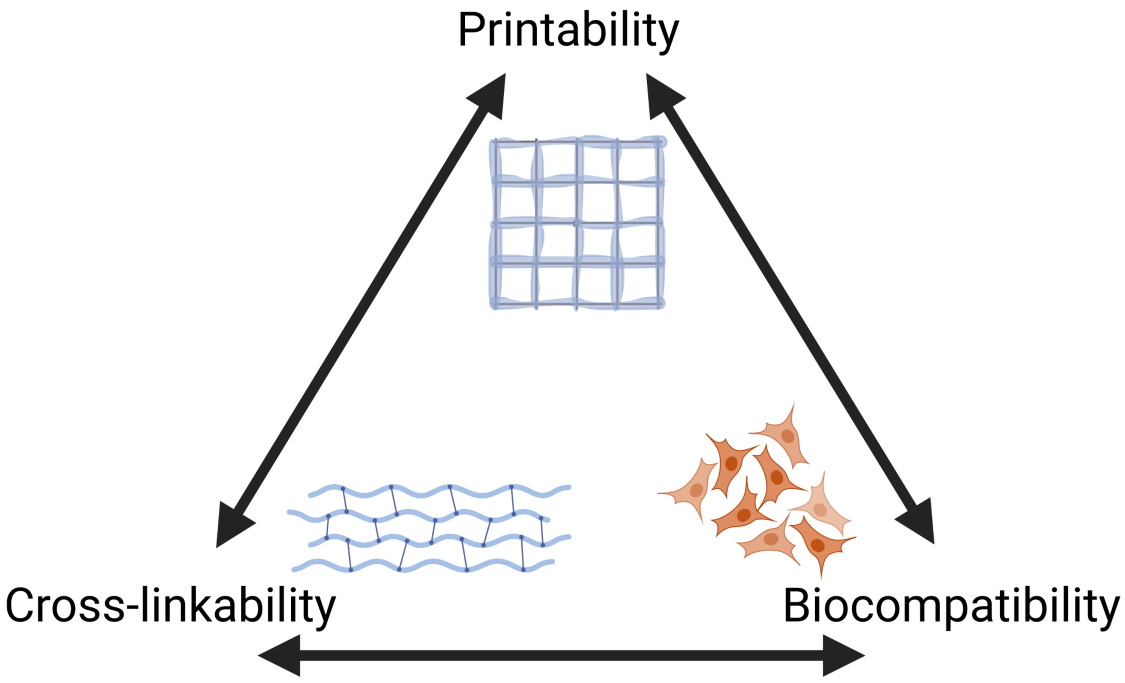

Fig. 4.1 Schematic representation of key characteristics of hydrogels for 3D printing in biological applications; Created with BioRender.com

These three specifications are interlinked and require considering factors such as rheological behavior, chemistry, mechanical stability, and morphology. However, there is an inverse relationship between printability and cell viability due to shear

stress-induced cell mortality [94], and harmfull effects of conventional UV cross-linking on cells [95], leading to the necessity of trade-offs in bioink development. Despite these and other challenges, like inadequate mechanical performance, the use of hydrogels as matrices for bioinks is promising.

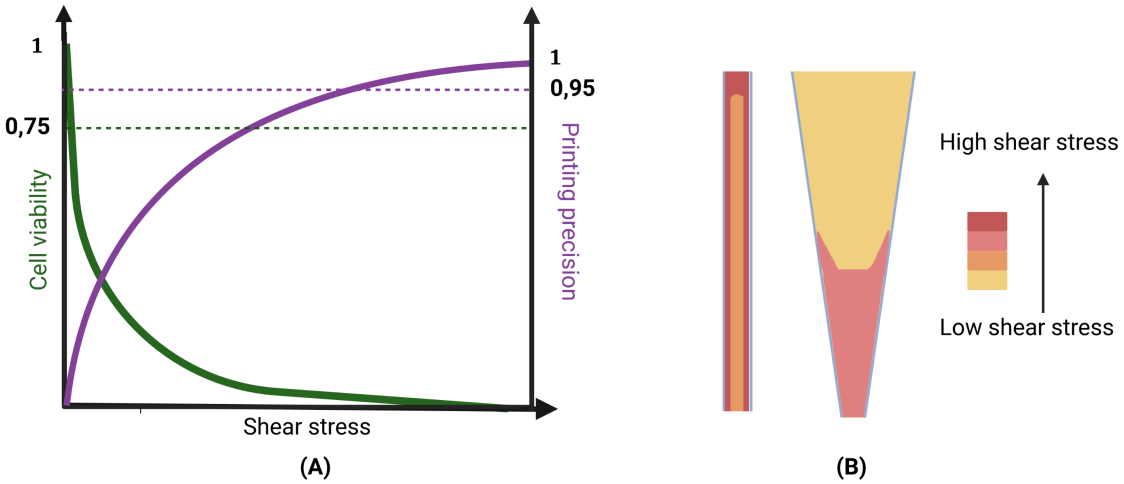

Fig. 4.2 ***(A)*** *Typical example of the influence of shear stress on printing precision and cell viability in microextrusion 3D printing with marked cell viability (75%) and printing precision (95%) thresholds (inspired by [96]);* ***(B)*** *Schematic representation of shear stress distribution in cylindrical and conical flow channel (adapted from [97]); Created with BioRender.com*

4.2.2 Cross-linking strategies

Hydrogel cross-linking can be generally done by the way of non-covalent (physical), or covalent (chemical) bonds. Non-covalent bonds are advantageous due to their reversibility, but generally show lower mechanical performance compared to non-reversible chemical cross-linking, although both types are widely utilized in 3D printable hydrogels [80].

Hydrogen bonds are prevalent in physical cross-linking in polymers like polyvinyl alcohol (PVA), gelatin, and agarose [98, 99, 100]. These bonds are thermally reversible with material dependent transition temperature. Another common non-covalent cross-linking strategy is metal ion complexation, which is useful especially for the cross-linking of anionic polymers (such as polysaccharides), where the addition of multivalent metal cations causes immediate gelation. In particular,

sodium alginate's cross-linking with calcium cations has been extensively studied [101, 102, 103, 104, 105]. Recent trends in non-covalently cross-linked hydrogels suggest supramolecular host-guest systems as promising for 3D printing in biomedical applications due to their dynamic, self-healing nature and swift response to external mechanical stimuli [106, 107].

Chemical cross-linking, on the other hand, typically involves low-molecular cross-linking agents. The most frequently used reactions take place at -OH, -NH_2, or -COOH groups. Hydroxyl groups, found in many natural and synthetic biocompatible polymers, can react with dialdehyde cross-linkers, e.g. glutaraldehyde or glyoxal [108, 109]. However, these low-molecular dialdehydes are known to induce oxidative stress in cells [110, 111], leading to efforts to find replacement in the form of naturally derived polyaldehydes [112].

Peptides and proteins, a large and diverse group of natural polymers, is rich in -NH_2 groups, which can form Schiff bases when exposed to aldehydes, leading to the so-called dynamic covalent bonds [113]. These bonds provide self-healing and shear-thinning hydrogels [114, 115], although they can be unstable in certain environments and require stabilization [116]. Acid anhydrides are another common reaction partner, particularly in methacrylation, a popular method for introducing photocross-linkability to natural polymers [117]. Although cross-linking via reactions of -NH_2 is especially convenient in case of proteins, many researchers introduce these functional groups to other polymer chains via carbodiimide chemistry in order to allow analogous cross-linking [118, 119].

Cross-linking based on carboxyl groups, foung e.g. in hyaluronan (HA) or chondroitin sulfate, requires activation of the reactive sites. The carbodiimide activating system, employing 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) and N-hydroxysuccinimide (NHS) or 1-hydroxybenzotriazole (HOBt) is often used. The reason is its versatility, which is demonstrated by the wide variety of outcomes provided by this reaction, including direct cross-linking via esterification of HA [120] or amidation [121], which can be used for functionalization with other reactive groups, including thiols [122, 123], or dienes and alkynes to enable Diels-Alder cycloaddition [124, 125]. Additionally, these reactions can allow for grafting polymers with cell adhesive molecules [126]. Despite the effectiveness of the EDC/NHS

(HOBt) system, the potential cytotoxicity due to the formation of stable N-acylurea has led to the exploration of alternatives, such as 4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride, which is highly efficient, water soluble, and lacks pH dependency [119].

4.3 Electrofluidodynamics

Electrofluidodynamics comprise phenomena occurring when a fluid is placed in an external electric field. In general, the external electric field causes imbalance of charges in the bulk and on the surface of the liquid, leading to electric pressure [127]. Once the pressure overcomes the capillary forces (i.e. surface tension), the repulsive electrostatic force induces formation of a Taylor cone [128] and subsequently liquid jet opposite to the direction of electric field gradient is formed. The electric pressure causing the initial instability of the liquid droplet arises from electric force, which can be found as:

$$F_e = \int (\frac{1}{2}\varepsilon E^2)\, ds, \tag{4.1}$$

where ε is the permittivity of the environment, and E is the electric field intensity. The electric field needs to be strong enough to overcome the capillary forces:

$$F_c = 2\pi r\gamma cos\theta, \tag{4.2}$$

where γ denotes the surface tension and θ is the contact angle between the liquid and the surface [4].

There are three major manufacturing technologies based on the electrofluidodynamic phenomena (Figure 4.4). First, it is the electrospraying technology, which is connected to the early observations of Rayleigh instability, or disintegration of the fluid jet into droplets [129]. This behaviour is driven by minimum energy principle,

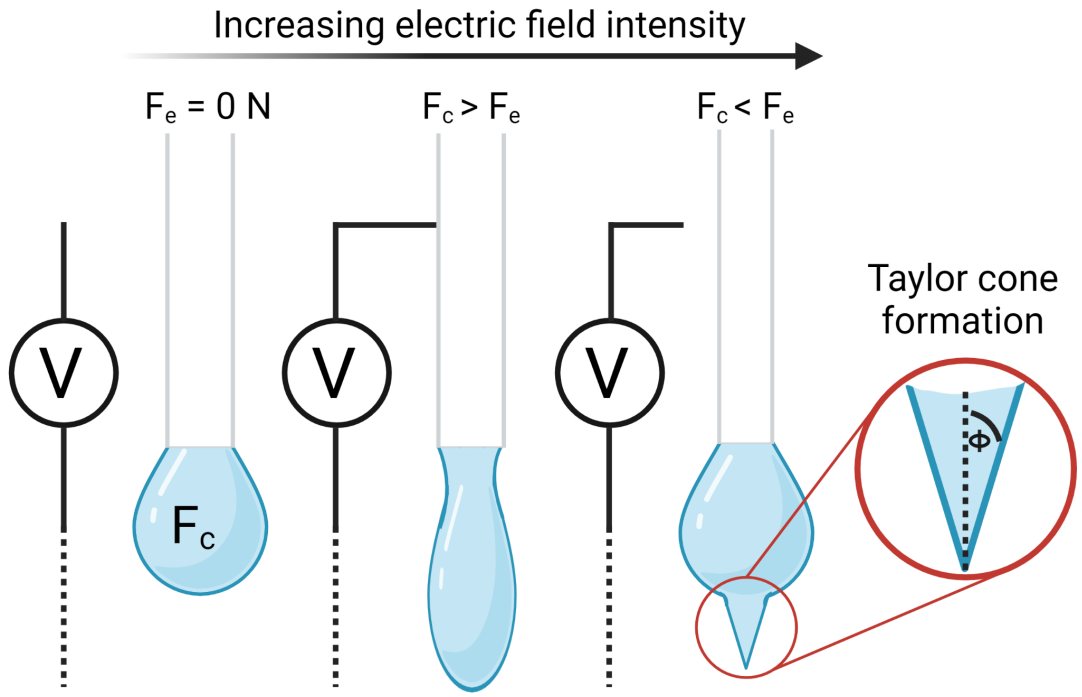

Fig. 4.3 Schematic representation of liquid droplet shape evolution with the increase of electric field intensity and Taylor cone formation (adapted from [4]); Created with BioRender.com

and depends on the balance of surface tension and electrostatic stabilization [130].

Electrowriting technology combines electrofluidodynamics and 3D printing principles, and takes place in the early jet path during stable phase, where viscoelastic forces dominate inertia [131]. Characterized by low elongation rates and a Trouton ratio (elongation to shear viscosity ratio) of 3, the technology capitalizes on a controlled spinneret move to position an electrically elongated jet [132]. The jet's stability is enhanced by strain hardening due to polymer chain entanglements, which makes branched or highly polydisperse polymers more advantageous in this sense [133].

Electrospinning, the main technique for nanofibre fabrication, begins once the polymer jet exceeds the stable region. The charged jet, constantly moving in an external electric field, develops a charge imbalance that bends the jet into an expanding coil, a phenomenon known as whipping instability [131]. This elongation significantly extends the jet path, as shown in Figure 4.4, reducing jet diameter and increasing its specific surface area, prompting swift solvent evaporation and fiber solidification in solution spinning [134].

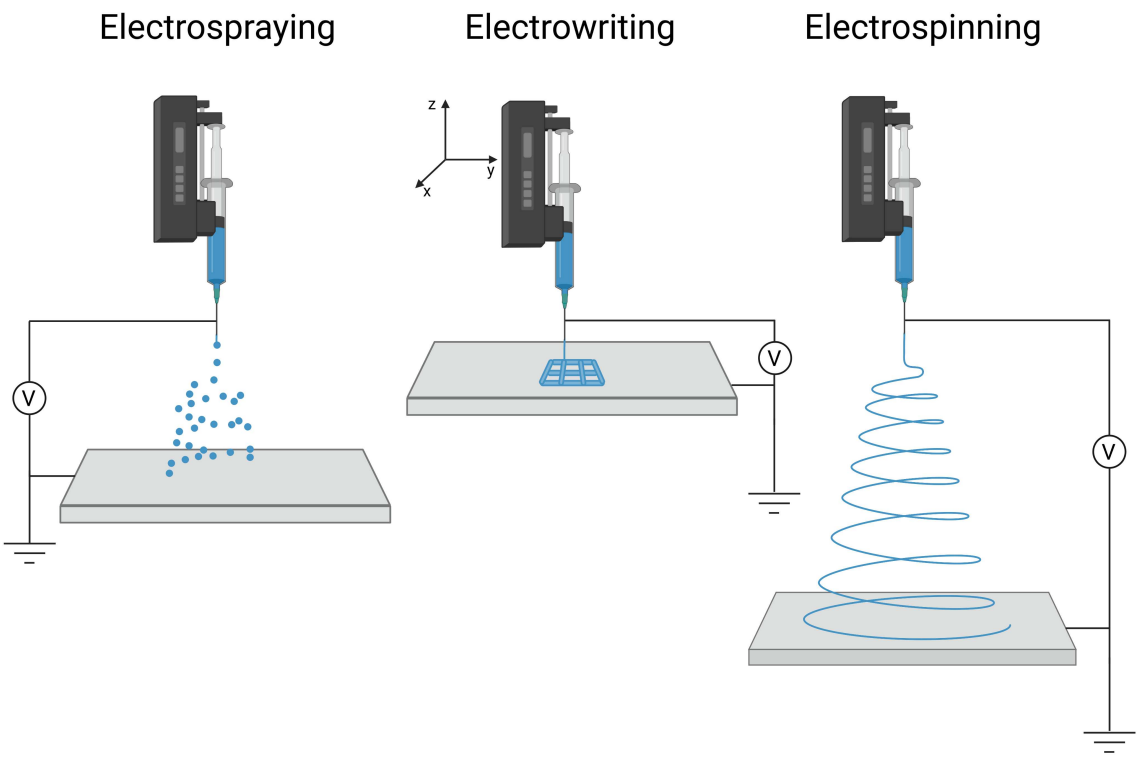

Fig. 4.4 Technologies based on electrofluidodynamic phenomena; Created with BioRender.com

4.3.1 Parameters influencing electrospinning

The electrospinning process as well as other electrofluidodynamic phenomena is influenced by number of parameters. These are usually divided into material, processing and ambient parameters - see Figure 4.5.

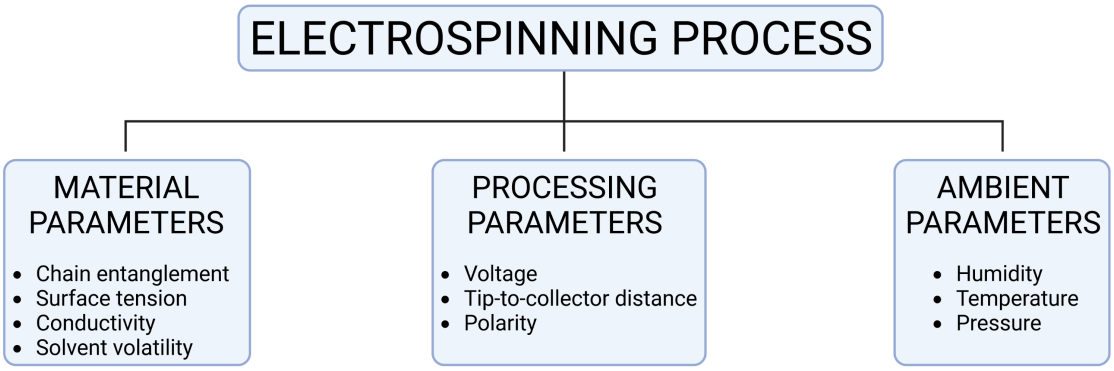

Fig. 4.5 Parameters influencing electrospinning; Created with BioRender.com

There are multiple solution parameters, which need to be taken into account with regards to electrospinning. The most outstanding role can be found for polymer chain entanglement, which involves multiple influences:

- polymer chain rigidity,
- polymer M_w,
- polymer-solvent interactions,
- polymer coil diameter.

Each of these parameters can be used to influence the outcome of the electrofluidodynamic fabrication. Shenoy et al., 2005 suggested 2,5 entanglements per chain as the minimum for overcoming initial Rayleigh instability [135]. This was contradicted by the findings of Malkin et al., 2017, who achieved stable jet spinning at lower entanglements due to stabilization effect of gradual solidification [134]. The interactions of the parameters are complex and lead to multiple effects on electrospinning process. The used solvent influences the polymer chain due to polymer-solvent interactions [136], surface tension [137], and evaporation rate, as well as polymer coil diameter [138]. Another means to change the conformation, especially in case of polar polymers, is addition of salts [139], which at the same time increases the conductivity of the solution. Higher conductivity leads to polymer jet stabilization against formation of beaded structure [140], and also possibility to form Taylor cones on the surface of emerging fibre, leading to branching [131].

The electric field intensity is directly proportional to the applied voltage, and inversely proportional to electrode - collector distance. Therefore, it is the central characteristic dictated by the processing parameters. However, short distance causes short jet path and limits the elongation zone and evaporation time. I may cause bead-on-string instabilities, or branching of the electrospun fibres [137, 141, 131, 142]. On the other hand, short jet path is essential for maintaining stable jet path, and allow electrowriting [132]. The electric field polarity is also reported as an important parameter influencing the surface energy of the electrospun fibres [143, 144]. While DC positive voltage remains the most popular option, DC negative or AC voltage can facilitate electrospinning as well [145, 146].

The straightforward understanding of humidity and temperature effects on electrofluidodynamic phenomena lies in their effects on evaporation rate, influencing the fibre diameter [147]. The specific results are highly dependent on the poly-

mer material, as increased humidity can increase [148] the fiber diameter, or cause the formation of bead-on string instability [149] in some cases. Furthermore, electrospinning of water-insoluble polymers in high humidity environment has been used to generate porous fibres *via* vapor-induced phase separation or breath figures method [150].

4.3.2 Core-shell electrospinning

Core-shell electrospun fibers are composites consisting of an inner fiber (core) enveloped by an outer material (shell). These structures can be exploited to tune various fibers properties and encapsulate low-molecular substances in the core [151]. Generally, core-shell fibers can be obtained either by co-axial or emulsion electrospinning [152]. Co-axial spinning employs a dual-channel spinneret for separate core and shell material flow [153], but shear stress generated at the core-shell interface can disrupt fiber integrity [154]. Thus, interfacial tension, arising from fluid miscibility and viscosity difference be taken into account. Conversely, emulsion electrospinning uses a single spinneret and immiscible liquid emulsion, creating core-shell fibers via rapid polymer jet stretching [152], where the continuous core is formed by electric field induced phase separation [155] or viscosity-driven enveloping [156].

4.3.3 Electric field assisted fabrication of scaffolds

Electrofluidodynamic processes result in constructs with large surface area, high porosity, and adjustable pore size [1]. Electrospraying dominates in drug delivery applications due to the particle-like structure and lack of spatial orientation [157, 158]. The spatial orientation imposes limitations also on electrospinning applications. While electrospinning can theoretically produce highly oriented structures, the whipping instability often disrupts fiber orientation, resulting in random fibrous mesh [159]. This is mitigated using specialized collectors [160, 161]. Nanofibrous scaffolds provide similarities to ECM, such as tunable porosity and mechanical characteristics, and aid in cell guidance through the presence of nanotopographical features [162]. Oriented nanofibrous structures lead to more aligned stem cell

cytoskeleton and enhanced adhesion [7, 39, 163, 164]. The superb control over positioning of thin strands in electrowriting, on the other hand, produces larger fibers that lack nanotopographical features [165]. Therefore, each technology can benefit certain medical applications in a unique way.

5 MAIN RESULTS OF THE THESIS

The results presented in the thesis can be divided into three subgroups:

1. printability of hydrogels achieved by dynamic covalent cross-linking,
2. printability of polymer solutions achieved by disc-like nanoparticulate rheological modifiers,
3. electrospinning of biopolymers into defectless fibres.

Dynamic covalent bonds, specifically Schiff bases, and the self-healing and shear-thinning hydrogels produced thereof, are outlined in **PAPER I**, **PAPER II**, and **PAPER III**. In research described in **PAPER I**, the utility of Schiff-base cross-linking was demonstrated by preparing hydrogels based on gelatin, with polysaccharide-based polyaldehyde used ass a less toxic alternative to bi-functional aldehydes. The study identified the source of gelatin and concentration of oxidized dextran as significant parameters influencing reaction rate, and importantly rheological properties.

Building upon this concept, the Schiff-base cross-linked HA hydrogel (Figure 5.2 **(A)** documents the shear thinning of the matrix) was considered a biocompatible matrix for encapsulating magnetic particles, thus providing magneto-responsive smart hydrogels. **PAPER II** describes the use of carbonyl iron particles (CIPs), which when present, led to considerable stiffening of the material in an external magnetic field through the MRE, as demonstrated in Figure 5.1 **(B) - (D)**. **PAPER III** on the other hand focused on the use of iron oxide multicore particles (MCPs) consisting of superparamagnetic iron oxide. In this case, the MRE is negligible.

However, when exposed to alternating magnetic field, these particles efficiently produce heat. By extension, the hydrogels filled with MCPs can serve as biocompatible heat mediators (see Figure 5.2 **(A)**). Furthermore, the hydrogel matrices were used to encapsulate mouse fibroblast and subject them to microextrusion. The process yielded over 80% cell viability, thus proving the capacity of the described hydrogel to provide a bioink, as can be seen in 5.2 **(C)** and **(D)**.

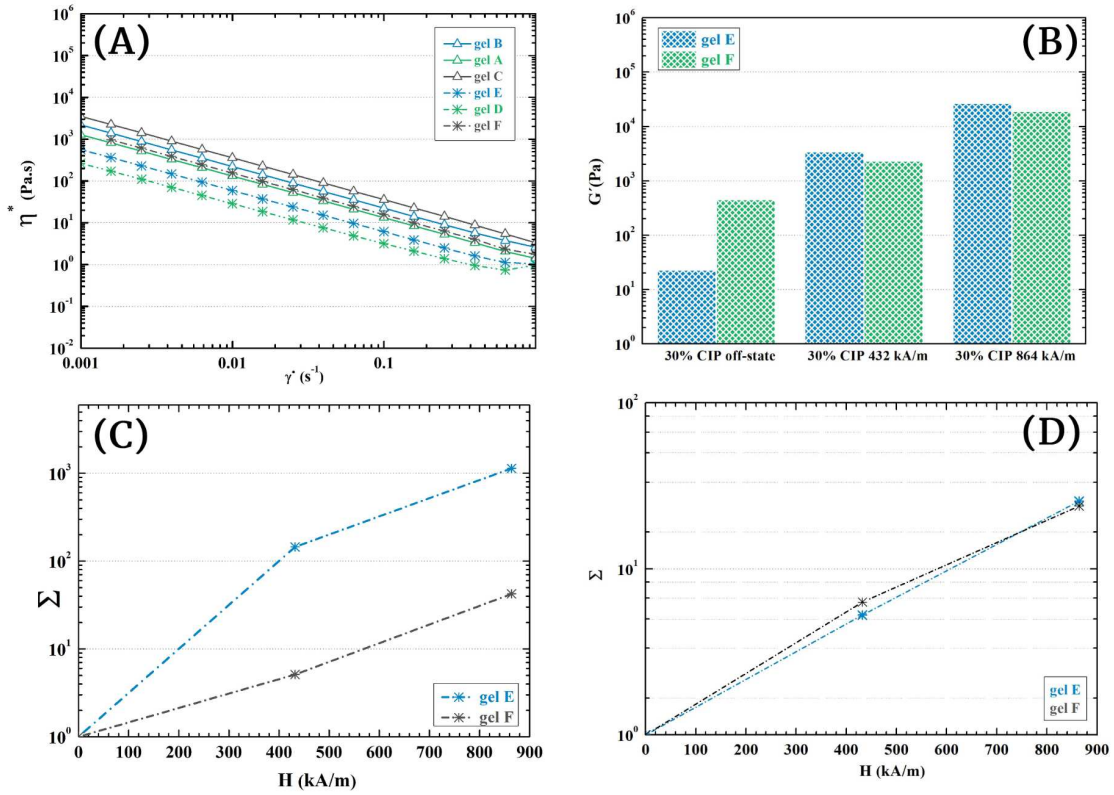

*Fig. 5.1 Magneto-responsive HA hydrogels with CIPs as a filler; gel A - HA-ADH DS 22% + HA-OX DO 35, gel B - HA-ADH DS 22% + HA-OX DO 62, gel C - HA-ADH DS 22% + DEX-OX DO 49, gel D - HA-ADH DS 12% + HA-OX DO 35, gel E - HA-ADH DS 12% + HA-OX DO 62, gel F - HA-ADH DS 12% + DEX-OX DO 49; **(A)** Dependence of viscosity on shear rate, **(B)** Storage modulus averaged over the stable region of shear rate - 0.01 s^{-1}-0.1 s^{-1} in an increasing magnetic field; **(C)** Magnitude of a storage modulus increase due to MRE relative to the initial value; **(D)** Magnitude of a storage modulus increase due to MRE relative to the initial value after hydrogels' stabilization with Fe^{3+} ions [166]*

However, an adverse effect of cross-linking *via* Schiff base formation in biocompatible hydrogels was observed, as their stability decreased significantly when exposed to standard cell cultivation conditions. The working hypothesis for this phe-

nomenon is the occurrence of a competitive reaction between the free amino-acids, which are a part of the cultivation medium, and aldehyde groups present on the oxidized polysaccharide chains, as opposed to the desired Schiff base formation with the -NH_2 containing polymers. Interestingly, the hydrogel decay was suppressed in hydrogels containing FeOx MCPs and $Al2O_3$ NPs, as shown in Figure 5.2 **(B)**. This observation was assumed to be connected to the the possible partial dissociation of Fe^{3+} and Al^{3+} cations, which have the capacity to form complexes with the polyanionic HA. Based on this assumption, the MRE displaying hydrogels were successfully stabilized with Fe^{3+} in **PAPER II**, while retaining a part of the original MRE.

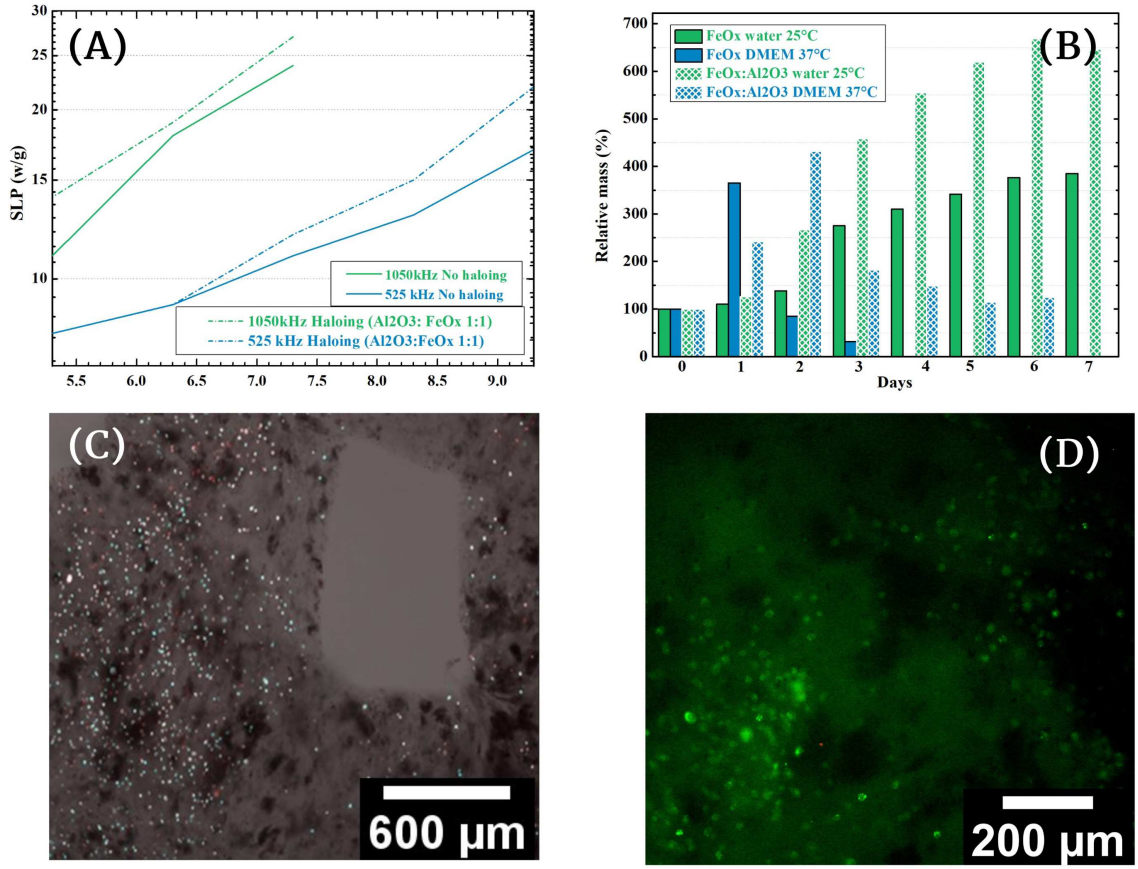

*Fig. 5.2 Magneto-responsive HA hydrogels with FeO_x MCPs and Al_2O_3 NPs as a filler; **(A)** Heating efficiency of the hydrogels expressed as the dependence of specific loss power (SLP) on alternating magnetic field amplitude; **(B)** Stability of hydrogels in water at 25°C and cultivation medium at 37°C; **(C)** Confocal fluorescence microscopy imaging of mouse fibroblasts distribution in 3D printed grid model; **(D)** live/dead assay of mouse fibroblasts encapsulated in the hydrogel and subjected to microextrusion, reaching over 80% cell viability*

In a different approach, organic-inorganic disc-like NPs were used as a rheological modifier for 3 wt.% sodium alginate (NaAlg) solutions. The rheological modification is based on the electrostatic repulsion of the charged NPs, leading to formation of random structure within the polymer matrix, so-called "house of cards" [61]. Due to the physical essence of the phenomenon, the "house of cards" is liable to shear stress. Thus, this phenomenon generally leads to increase of viscosity in steady state, while in shear flow the viscosity decreases, and the materials displays shear thinning behaviour. **PAPER IV** demonstrated that the obtained rheological profile enhances 3D printability via microextrusion in low-viscosity polymer solutions (Figure 5.3 **(A)**). The random orientation of the particles was observed by AFM, see Figure 5.3 **(B)**, which is in agreement with the "house-of-cards" formation hypothesis. Additionally, it was proved that the hydrogels can serve as bioinks by encapsulating of mouse fibroblasts in the material, followed by 3D printing and live/dead assay shown in Figure 5.3 **(D)**.

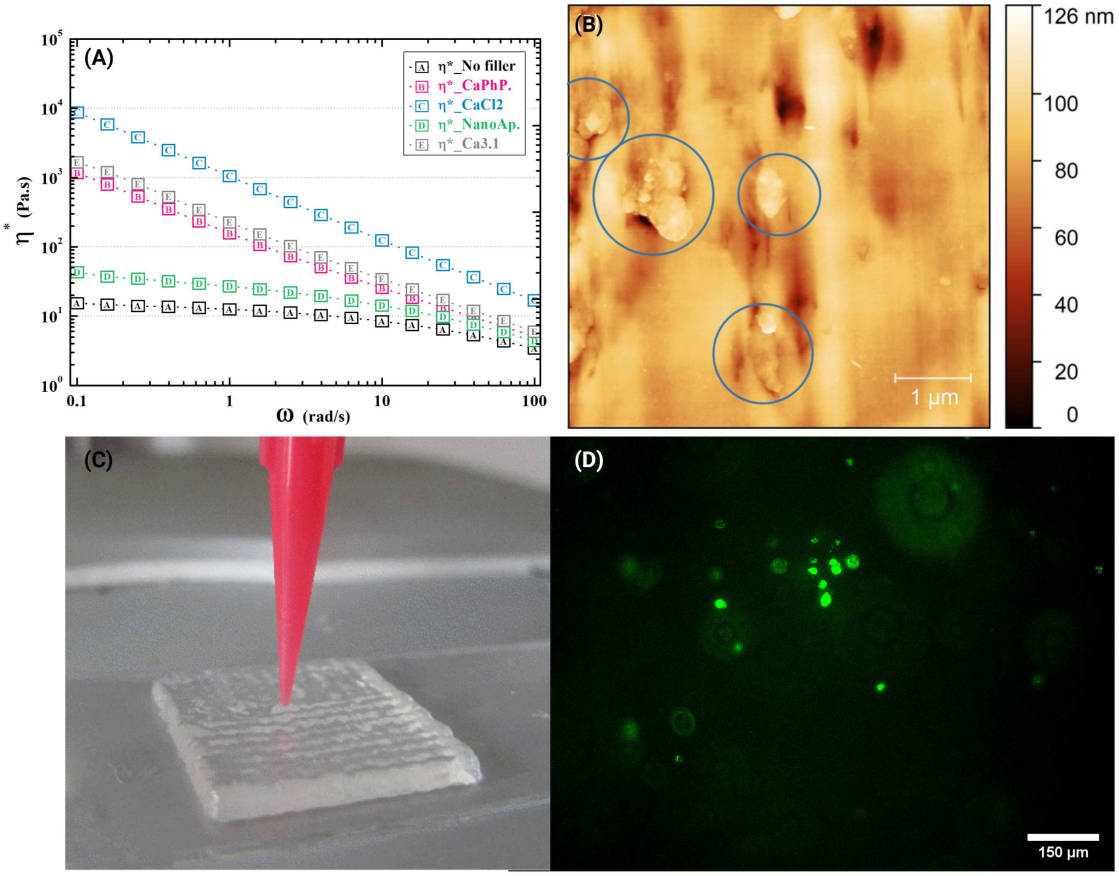

Fig. 5.3 ***(A)*** *Dependence of viscosity on angular frequency as measured for NaAlg solution in pure state and with various fillers: exfoliated layered CaPhP and Ca3.1, spherical NanoAp, free* Ca^{2+} *ions in the form of* $CaCl_2$ *ethylene glycol solution;* ***(B)*** *atomic force microscopy micrographs of randomly oriented layered particles in the NaAlg;* ***(C)*** *example of 3D printing of layered NPs filled NaAlg solution via microextrusion;* ***(D)*** *live/dead assay of mouse fibroblasts encapsulated in the layered NPs containing NaAlg and subjected to microextrusion, reaching at least 75% cell viability [167]*

PAPER V addressed the preparation of HA-based nanofibrous structures using electrospinning. In order to achieve this, two strategies were applied - co-electrospinning with biocompatible PVA or polyethylene oxide (PEO), and the use of intermediate solvent. While both strategies facilitated fibre formation, polymer co-electrospinning resulted in beads-on-string structured fibers. Conversely, the choice of an appropriate intermediate solvent - a mixture of water and alcohols - facilitated the electrospinning of smooth, defectless fibres, as evidenced by scanning electron microscopy micrographs in Figure 5.4 **(A)** and **(B)**. The research of electrospun fibers was extended towards core-shell fibers, with biocompatible water-insoluble poly-ε-

caprolactone (PCL) used as the shell material, and natural polymers - collagen or HA - as the core. An example of a successful core-shell structured fibre is presented in Figure 5.4 **(C)**. Nevertheless, further research is necessary to fully understand and tune these processes.

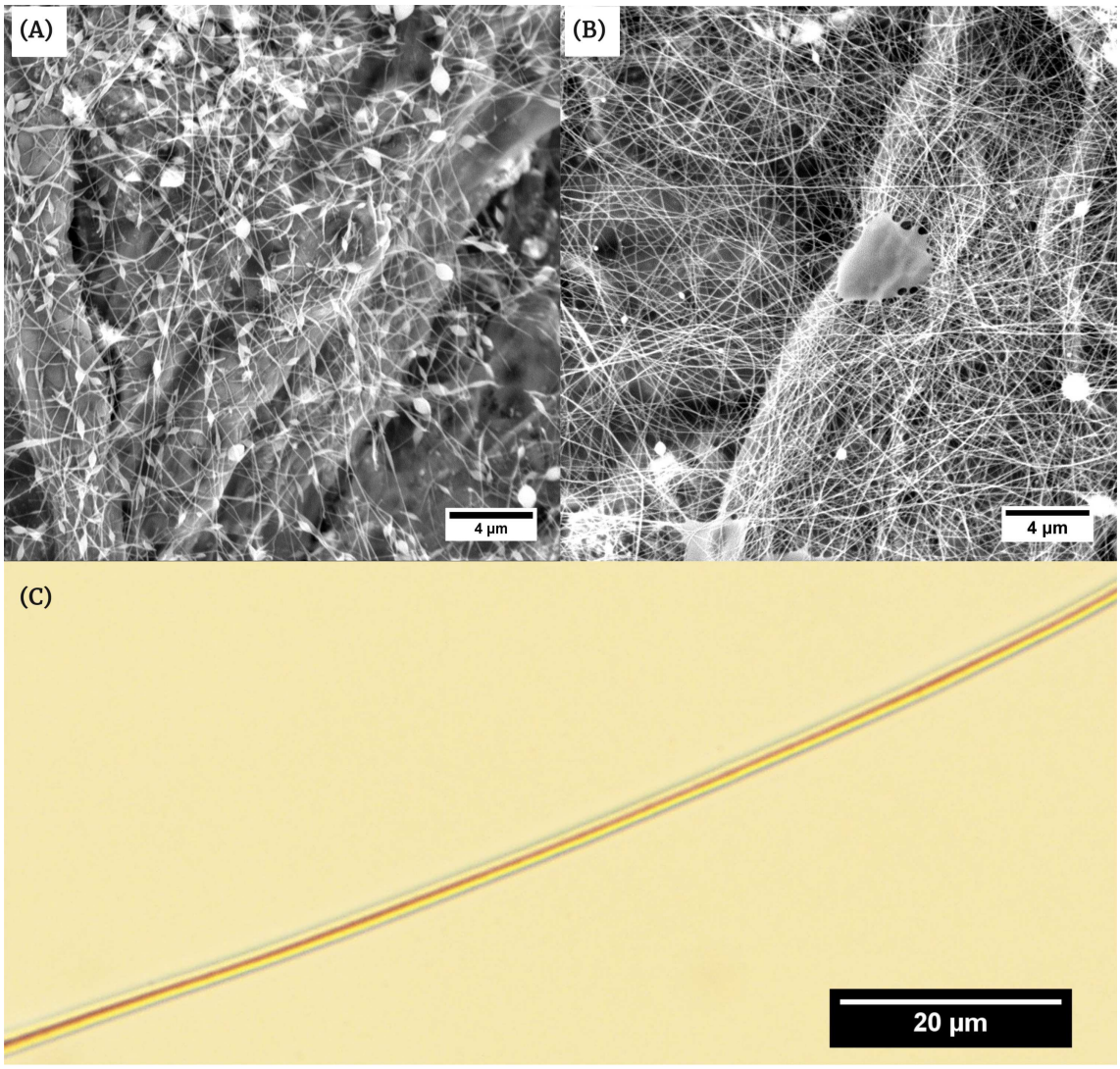

Fig. 5.4 Scanning electron microscopy micrographs of ***(A)*** *HA-PEO blend electrospun bead-on-string structured fibres;* ***(B)*** *HA nanofibres spun from intermediate solvent (H_2O:EtOH:MeOH 5:5:1 mixture) [168];* ***(C)*** *polarized light optical microscopy micrograph of collagen-PCL core-shell electrospun fibre*

6 CONTRIBUTION TO RESEARCH AND PRACTICE

This study aims to enhance the usability of biopolymers in advanced processing technologies - 3D printing and electrospinning. There are fundamental differences in the technologies. Notably, 3D printing mainly involves shear stress, while electrospinning produces elongation flow. In terms of materials, both natural and synthetic polymers can be employed in these applications. However, overall the work aims towards materials use in medical applications, thus, non-cytotoxicity is essential.

The primary characteristic for 3D printing is the rheological profile, requiring shear-thinning materials and fast recovery upon lifting of the shear stress. Such behaviour can be achieved with dynamic polymer networks, e.g. Schiff base cross-linking, or electrostatically driven layered NPs formed "house-of-cards" structure.

Electrospinning in general relies on multiple intrinsic and extrinsic factors, with specific peculiarities taking place in core-shell electrospinning. The complex relationships are examined in order to achieve better understanding of the electrofluidodynamic phenomena and allow the transfer of the acquired knowledge to practice.

The thesis gives the foundation for preparing advanced cell culture scaffolds by combining the precise 3D printed structures with electrospinning-provided nanofeatures. These can be structured in many different forms with specific advantages, such as nanofibres decorated 3D printed structures [169, 170], layered 3D printed-electrospun sandwich structured scaffolds [171], and nanofibres-reinforced 3D printing inks [172] designed to enhance cell adhesion, proliferation, and potentially morphogenesis.

7 CONCLUSION

The thesis focuses on enhancing the usability of biopolymers in advanced processing technologies - 3D printing and electrospinning, to obtain precisely structured

scaffolds with distinct macro-, micro- and nanostructures. The work emphasises potential advantages for medical applications. In terms of 3D printing, the hydrogel needs to provide shear-thinning rheological profile. The thesis described the rheological modification by employing dynamic polymer networks, either dynamic covalent cross-linking *via* Schiff base formation, or electrostatically driven layered NPs "house of cards" supportive structure. Both approaches provided shear-thinning inks suitable for 3D printing *via* microextrusion with satisfactory shape fidelity. Moreover, the hydrogels allow encapsulation of living cells and their subsequent microextrusion based 3D printing with sufficient cell viability >75%. Therefore, the developed materials have the capacity to form bioinks. Additionally, the Schiff base cross-linked hydrogels were tested as matrices for magneto-responsive particles, making them promising candidates in preparation of smart hydrogels for bioapplications. Furthermore, the document discusses successful strategies for electrospinning biopolymers into defectless fibers. The electrospinning of HA *via* the use of intermediate solvent was found superior to the conventional strategy of polymer co-electrospinning, as it allows fabrication of smooth fibers of 50-20 nm in diameter. To widen the usability of the natural polymer based fibers, core-shell fibers enveloping natural polymer (HA or Gel) in biocompatible PCL were prepared. Combining the precision layer-by-layer fabrication with nanofeatures provided by electrospun fibers outlined in the thesis provides tools for developing advanced cell culture scaffolds.

REFERENCES

[1] LAURENCIN, C. and NAIR, L. *Nanotechnology and Regenerative Engineering: The Scaffold - 2. edition*. CRC Press, 2015. ISBN 978-1-138-07658-7.

[2] PRAKASH, K., NANCHARAIH, T. and RAO, V. S. Additive Manufacturing Techniques in Manufacturing -An Overview. *Materials Today: Proceedings*. 2018, 5, pp. 3873–3882. doi: 10.1016/j.matpr.2017.11.642.

[3] NOH, I., KIM, N., TRAN, H., LEE, J. and LEE, C. 3D printable hyaluronic acid-based hydrogel for its potential application as a bioink in tissue engineering. *Biomaterials Research*. 2019, 23. doi: 10.1186/s40824-018-0152-8.

[4] LUKÁŠ, D., SARKAR, A., MARTINOVÁ, L., VODSEĎÁLKOVÁ, K., LUBASOVÁ, D., CHALOUPEK, J., POKORNÝ, P., MIKEŠ, P., CHVOJKA, J. and KOMÁREK, M. Physical principles of electrospinning (electrospinning as a nano-scale technology of the twenty-first century). *Text. Prog.* 2009, 41, pp. 59–140. doi: 10.1080/00405160902904641.

[5] MORI, A. D., FERNÁNDEZ, M. P., BLUNN, G., TOZZI, G. and ROLDO, M. 3D Printing and Electrospinning of Composite Hydrogels for Cartilage and Bone Tissue Engineering. *Polymers*. 2018, 10, pp. 285–311. doi: 10.3390/polym10030285.

[6] TIBBITT, M. and ANSETH, K. Hydrogels as Extracellular Matrix Mimics for 3D Cell Culture. *Biotechnology and Bioengineering*. 2009, 103. doi: 10.1002/bit.22361.

[7] DE FRANCE, K., XU, F., TOUFANIAN, S., CHAN, K., SAID, S., STIMPSON, T., GONZALEZ-MARTINEZ, E., MORAN-MIRABAL, J., CRANSTON, E. and HOARE, T. Multi-scale structuring of cell-instructive cellulose nanocrystal composite hydrogel sheets via sequential electrospinning and thermal wrinkling. *Acta Biomaterialia*. 2021, 128. doi: 10.1016/j.actbio.2021.04.044.

[8] MEI, L., WANG, Y., TONG, A. and GUO, G. Facile electrospinning of an efficient drug delivery system. *Expert Opinion on Drug Delivery*. 2016, 13. doi: 10.1517/17425247.2016.1142525.

[9] NANGARE, S., JADHAV, N., GHAGARE, P. and MUTHANE, T. Pharmaceutical applications of electrospinning. *Pharmaceutiques Françaises*. 2020, 78. doi: 10.1016/j.pharma.2019.07.002.

[10] KHAN, A., MORSI, Y., ZHU, T., AHMAD, A., XIE, X., YU, F. and MO, X. Electrospinning: An emerging technology to construct polymer-based nanofibrous scaffolds for diabetic wound healing. *Frontiers of Materials Science*. 2021, 15. doi: 10.1007/s11706-021-0540-1.

[11] KONG, B., R. LIU, J.H. GUO, L. LU and ZHAO, Q. Tailoring micro/nanofibers for biomedical applications. *Bioactive Materials*. 2023, 19. doi: 10.1016/j.bioactmat.2022.04.016.

[12] CURTI, F., A. SERAFIM, E. OLARET, S. DINESCU, I. SAMOILA, B.S. VASILE, H. IOVU, A. LUNGU, I.C. STANCU and R. MARINESCU. Development of Biocomposite Alginate-Cuttlebone-Gelatin 3D Printing Inks Designed for Scaffolds with Bone Regeneration Potential. *Marine Drugs*. 2022, 20. doi: 10.3390/md20110670.

[13] GAO, J., YU, X., WANG, X., HE, Y. and DING, J. Biomaterial–Related Cell Microenvironment in Tissue Engineering and Regenerative Medicine. *Engineering*. 2022, 13. doi: 10.1016/j.eng.2021.11.025.

[14] PAMPALONI, F., REYNAUD, E. and STELZER, E. The third dimension bridges the gap between cell culture and live tissue. *Nature Reviews Molecular Cell Biology*. 2007, 8, pp. 839–845. doi: 10.1038/nrm2236.

[15] LI, C., C.W. LI, Z.J. MA, H.F. CHEN, H.T. RUAN, J.F. DENG, J.W. WANG and W.G. CUI. Regulated macrophage immune microenvironment in 3D printed scaffolds for bone tumor postoperative treatment. *Bioactive Materials*. 2023, 19. doi: 10.1016/j.bioactmat.2022.04.028.

[16] BERTELS, S. et al. Geometrically defined environments direct cell division rate and subcellular YAP localization in single mouse embryonic stem cells. *Scientific Reports*. 2021, 11. doi: 10.1038/s41598-021-88336-y.

[17] ZHAO, F., ZHANG, C., LIU, J., LIU, L., CAO, X., CHEN, X., LEI, B. and SHAO, L. Periosteum structure/function-mimicking bioactive scaffolds with

piezoelectric/chem/nano signals for critical-sized bone regeneration. *Chemical Engineering Journal*. 2020, 402. doi: 10.1016/j.cej.2020.126203.

[18] MARIANO, A., BOVIO, C., CRISCUOLO, V. and SANTORO, F. Bioinspired micro- and nano-structured neural interfaces. *Nanotechnology*. 2022, 33. doi: 10.1088/1361-6528/ac8881.

[19] SCHAFFLER, M. and BURR, D. Stiffness of compact bone: Effects of porosity and density. *Journal of Biomechanics*. 1988, 21. doi: 10.1016/0021-9290(88)90186-8.

[20] SINGH, G. and CHANDA, A. Mechanical properties of whole-body soft human tissues: a review. *Biomedical Materials*. 2021, 16. doi: 10.1088/1748-605X/ac2b7a.

[21] SUTRADHAR, A. and MILLER, M. In vivo measurement of breast skin elasticity and breast skin thickness. *Skin Research and Technology*. 2013, 19. doi: 10.1111/j.1600-0846.2012.00627.x.

[22] STANTON, A., TONG, X., JING, S., BEHN, A., STORACI, H. and YANG, F. Aligned Gelatin Microribbon Scaffolds with Hydroxyapatite Gradient for Engineering the Bone–Tendon Interface. *Tissue Engineering Part A*. 2022, 28, pp. 712–723. doi: 10.1089/ten.tea.2021.0099.

[23] BANDYOPADHYAY, J. and SINHA, R. *Advances in Polymer Nanocomposites*, Clay-containing poly(ethylene terephthalate) (PET)-based polymer nanocomposites. Elsevier, 2012. ISBN 9781845699406.

[24] DÍAZ-PAYNO, P., KALOGEROPOULOU, M., MUNTZ, I., KINGMA, E., KOPS, N., D'ESTE, M., KOENDERINK, G., FRATILA-APACHITEI, L., OSCH, G. and ZADPOOR, A. Swelling-Dependent Shape-Based Transformation of a Human Mesenchymal Stromal Cells-Laden 4D Bioprinted Construct for Cartilage Tissue Engineering. *Advanced Healthcare Materials*. 2023, 12. doi: 10.1002/adhm.202201891.

[25] ZHAO, L., FENG, Z., LYU, Y., YANG, J., LIN, L., BAI, H., LI, Y., FENG, Y. and CHEN, Y. Electroactive injectable hydrogel based on oxidized sodium al-

ginate and carboxymethyl chitosan for wound healing. *International Journal of Biological Macromolecules*. 2023, 230. doi: 10.1016/j.ijbiomac.2023.123231.

[26] KO, E., KIM, C., CHOI, Y. and LEE, K. 3D printing of self-healing ferrogel prepared from glycol chitosan, oxidized hyaluronate, and iron oxide nanoparticles. *Carbohydrate Polymers*. 2020, 245. doi: 10.1016/j.carbpol.2020.116496.

[27] SHOU, Y. et al. Mechano-responsive hydrogel for direct stem cell manufacturing to therapy. *Bioactive Materials*. 2023, 24, pp. 387–400. doi: 10.1016/j.bioactmat.2022.12.019.

[28] GHOLAMIPOUR-SHIRAZI, A., NORTON, I. and MILLS, T. Dual stimuli-sensitive carrageenan-based formulation for additive manufacturing. *International Journal of Biological Macromolecules*. 2021, 189, pp. 370–379. doi: 10.1016/j.ijbiomac.2021.08.127.

[29] KAMPERMAN, T., WILLEMEN, N., KELDER, C., KOERSELMAN, M., BECKER, M., LINS, L., JOHNBOSCO, C., KARPERIEN, M. and LEIJTEN, J. Steering Stem Cell Fate within 3D Living Composite Tissues Using Stimuli-Responsive Cell-Adhesive Micromaterials. *Advanced Science*. 2023. doi: 10.1002/advs.202205487.

[30] STEVENS, M. and GEORGE, J. Exploring and engineering the cell surface interface. *Science*. 2005, 310, pp. 1135–1138. doi: 10.1126/science.1106587.

[31] DISCHER, D., MOONEY, D. and ZANDSTRA, P. Growth factors, matrices, and forces combine and control stem cells. *Science*. 2009, 324, pp. 1673–1677. doi: 10.1126/science.1171643.

[32] MITRAGOTRI, S. and LAHANN, J. Physical approaches to biomaterial design. *Nature Materials*. 2009, 8, pp. 15–23. doi: 10.1038/nmat2344.

[33] PLACE, E., EVANS, N. and STEVENS, M. Complexity in biomaterials for tissue engineering. *Nature Materials*. 2009, 8, pp. 457–470. doi: 10.1038/nmat2441.

[34] NEL, A., MÄDLER, L., VELEGOL, D., XIA, T., HOEK, E., SOMASUNDARAN, P., KLAESSIG, F., CASTRANOVA, V. and THOMPSON, M. Understanding bio-

physicochemical interactions at the nano–bio interface. *Nature Materials*. 2009, 8, pp. 543–557. doi: 10.1038/nmat2442.

[35] SONG, Y., ZHANG, Y., QU, Q., ZHANG, X., LU, T., XU, J., MA, W., ZHU, M., HUANG, C. and XIONG, R. Biomaterials based on hyaluronic acid, collagen and peptides for three-dimensional cell culture and their application in stem cell differentiation. *International Journal of Biological Macromolecules*. 2023, 226, pp. 14–36. doi: 10.1016/j.ijbiomac.2022.11.213.

[36] BISSELL, M., RIZKI, A. and MIAN, I. Tissue architecture: the ultimate regulator of breast epithelial function. *Current Opinion in Cell Biology*. 2003, 15, pp. 753–762. doi: 10.1016/j.ceb.2003.10.016.

[37] ZIELIŃSKI, P., GUDETI, P., RIKMANSPOEL, T. and WŁODARCZYK-BIEGUN, M. 3D printing of bio-instructive materials: Toward directing the cell. *Bioactive Materials*. 2023, 19, pp. 292–327. doi: 10.1016/j.bioactmat.2022.04.008.

[38] FORNETTI, E. et al. A novel extrusion-based 3D bioprinting system for skeletal muscle tissue engineering. *Biofabrication*. 2023, 15. doi: 10.1088/1758-5090/acb573.

[39] CHEN, Y., CHAN, J., WU, J., LI, R. and SANTERRE, J. Compatibility and function of human induced pluripotent stem cell derived cardiomyocytes on an electrospun nanofibrous scaffold, generated from an ionomeric polyurethane composite. *Journal of Biomedical Materials Research Part A*. 2022, 110, pp. 1932–1943. doi: 10.1002/jbm.a.37428.

[40] MANCINO, C., HENDRICKSON, T., WHITNEY, L., PARADISO, F., ABASI, S., TASCIOTTI, E., TARABALLI, F. and GUISEPPI-ELIE, A. Electrospun electroconductive constructs of aligned fibers for cardiac tissue engineering. *Nanomedicine: Nanotechnology, Biology and Medicine*. 2022, 44. doi: 10.1016/j.nano.2022.102567.

[41] ADADI, N., YADID, M., GAL, I., ASULIN, M., FEINER, R., EDRI, R. and DVIR, T. Electrospun Fibrous PVDF-TrFe Scaffolds for Cardiac Tissue Engineering, Differentiation, and Maturation. *Advanced Materials Technologies*. 2020, 5. doi: 10.1002/admt.201900820.

[42] ZHANG, J., CHERAGA, N. and HUANG, N. Biointerphases. *3D cell/scaffold model based on aligned-electrospun-nanofiber film/hydrogel multilayers for construction of anisotropic engineered tissue*. 2022, 17. doi: 10.1116/6.0002058.

[43] GONZALEZ-PUJANA, A., CARRANZA, T., SANTOS-VIZCAINO, E., IGARTUA, M., GUERRERO, P., HERNANDEZ, R. and CABA, K. Hybrid 3D Printed and Electrospun Multi-Scale Hierarchical Polycaprolactone Scaffolds to Induce Bone Differentiation. *Pharmaceutics*. 2022, 14. doi: 10.3390/pharmaceutics14122843.

[44] ALKAISSY, R., RICHARD, M., MORRIS, H., SNELLING, S., PINCHBECK, H., CARR, A. and MOUTHUY, P. Manufacture of Soft-Hard Implants from Electrospun Filaments Embedded in 3D Printed Structures. *Macromolecular Bioscience*. 2022, 22. doi: 10.1002/mabi.202200156.

[45] MATYJASZEWSKI, K. and MÖLLER, M. *Polymer Science: A Comprehensive Reference*. Elsevier Science, 2012. ISBN 978-0-08-087862-1.

[46] WISTRAND, A. and ALBERTSSON, A. Tuned mechanical properties achieved by varying polymer structure - Knowledge that generates new materials for tissue engineering. *Chinese Journal of Polymer Science*. 2007, 25. doi: 10.1142/S025676790700190X.

[47] CZARNECKI, S., ROSSOW, T. and SEIFFERT, S. Hybrid Polymer-Network Hydrogels with Tunable Mechanical Response. *Polymers*. 2016, 8. doi: 10.3390/polym8030082.

[48] RUYMBEKE, E., LEE, H., CHANG, T., NIKOPOULOU, A., HADJICHRISTIDIS, N., SNIJKERS, F. and VLASSOPOULOS, D. Molecular rheology of branched polymers: decoding and exploring the role of architectural dispersity through a synergy of anionic synthesis, interaction chromatography, rheometry and modeling. *Soft Matter*. 2014, 10. doi: 10.1039/c4sm00105b.

[49] XU, S., LIU, J. and WANG, X. Thermal Conductivity Enhancement of Polymers via Structure Tailoring. *Journal of Enhanced Heat Transfer*. 2020, 27. doi: 10.1615/JEnhHeatTransf.2020034592.

[50] RODRIGUEZ, F., COHEN, C., OBER, C. and ARCHER, L. *Principles of Polymer Systems - 6th edition*. CRC Press, 2015. ISBN 78-1-4822-2379-8.

[51] ABDOLLAHIYAN, P., OROOJALIAN, F. and MOKHTARZADEH, A. The triad of nanotechnology, cell signalling, and scaffold implantation for the successful repair of damaged organs: An overview on soft-tissue engineering. *Journal of Controlled Release*. 2021, 332. doi: 10.1016/j.jconrel.2021.02.036.

[52] ADEL, I., ELMELIGY, M. and ELKASABGY, N. Conventional and Recent Trends of Scaffolds Fabrication: A Superior Mode for Tissue Engineering. *Pharmaceutics*. 2022, 14. doi: 10.3390/pharmaceutics14020306.

[53] OZDIL, D. and AYDIN, H. Polymers for medical and tissue engineering applications. *Journal of Chemical Technology and Biotechnology*. 2014, 89. doi: 10.1002/jctb.4505.

[54] KATO, R., KAGA, C., KUNIMATSU, M., KOBAYASHI, T. and HONDA, H. Peptide array-based interaction assay of solid-bound peptides and anchorage-dependant cells and its effectiveness in cell-adhesive peptide design. *Journal of Bioscience and Bioengineering*. 2006, 101, pp. 485–495. doi: 10.1263/jbb.101.485.

[55] CHIMENE, D., LENNOX, K., KAUNAS, R. and GAHARWAR, A. Advanced Bioinks for 3D Printing: A Materials Science Perspective. *Annals of Biomedical Engineering*. 2016, 44, pp. 2090–2102. doi: 10.1007/s10439-016-1638-y.

[56] SOMIYA, S., YOSHIMURA, R. and WATANABE, R. *Sintering '87*. Springer, 1987.

[57] ESTELLE, K., BLAIR, D., EVANS, K. and GOZEN, B. Manufacturing of smart composites with hyperelastic property gradients and shape memory using fused deposition. *Journal of Manufacturing Processes*. 2017, 28, pp. 500–507. doi: 10.1016/j.jmapro.2017.04.018.

[58] SUN, W., TASHMAN, J., SHIWARSKI, D., FEINBERG, A. and WEBSTER-WOOD, V. Long-Fiber Embedded Hydrogel 3D Printing for Structural Reinforcement. *ACS Biomaterials Science Engineering*. 2022, 8, pp. 303–313. doi: 10.1021/acsbiomaterials.1c00908.

[59] CAI, Y., CHANG, S., GAN, S., MA, S., LU, W. and YEN, C. Nanocomposite bioinks for 3D bioprinting. *Acta Biomaterialia*. 2022, 151, pp. 45–69. doi: 10.1016/j.actbio.2022.08.014.

[60] BHATTACHARYYA, A., JANARTHANAN, G. and NOH, I. Nano-biomaterials for designing functional bioinks towards complex tissue and organ regeneration in 3D bioprinting. *Additive Manufacturing*. 2021, 37. doi: 10.1016/j.addma.2020.101639.

[61] DÁVILA, J. and D'ÁVILA, M. Rheological evaluation of Laponite/alginate inks for 3D extrusion-based printing. *The International Journal of Advanced Manufacturing Technology*. 2019, 101, pp. 675–686. doi: 10.1007/s00170-018-2876-y.

[62] BOSSIS, G., VOLKOVA, O., LACIS, S. and MEUNIER, A. *Ferrofluids. Lecture Notes in Physics*, Magnetorheology: Fluids, Structures and Rheology. Springer: Berlin, Germany, 2022.

[63] LIU, J., FLORES, G. A. and SHENG, R. In-vitro investigation of blood embolization in cancer treatment using magnetorheological fluids. *Journal of Magnetism and Magnetic Materials*. 2001, 225, pp. 209–217. doi: 10.1016/S0304-8853(00)01260-9.

[64] TAY, A., SOHRABI, A., POOLE, K., SEIDLITS, S. and CARLO, D. D. A 3D magnetic hyaluronic acid hydrogel for magnetomechanical neuromodulation of primary dorsal root ganglion neurons. *Advanced Materials*. 2018, 30. doi: 10.1002/adma.201800927.

[65] SHARMA, S., SHRIVASTAVA, N., ROSSI, F., TUNG, L. and THANH, N. Nanoparticles-based magnetic and photo induced hyperthermia for cancer treatment. *Nano Today*. 2019, 29. doi: 10.1016/j.nantod.2019.100795.

[66] OTA, S. and TAKEMURA, Y. Characterization of Néel and Brownian Relaxations Isolated from Complex Dynamics Influenced by Dipole Interactions in Magnetic Nanoparticles. *The Journal of Physical Chemistry C*. 2019, 123. doi: 10.1021/acs.jpcc.9b06790.

[67] DUTZ, S. and HERGT, R. Magnetic particle hyperthermia—a promising tumour therapy? *Nanotechnology*. 2014, 25. doi: 10.1088/0957-4484/25/45/452001.

[68] CHEN, R., ROMERO, G., CHRISTIANSEN, M., MOHR, A. and ANIKEEVA, P. Wireless magnetothermal deep brain stimulation. *Science*. 2015, 347. doi: 10.1126/science.1261821.

[69] VANGIJZEGEM, T., STANICKI, D. and LAURENT, S. Magnetic iron oxide nanoparticles for drug delivery: applications and characteristics. *Expert Opinion on Drug Delivery*. 2019, 16. doi: 10.1080/17425247.2019.1554647.

[70] WANG, J., ZHANG, Y., AGHDA, N., PILLAI, A., THAKKAR, R., NOKHODCHI, A. and MANIRUZZAMAN, M. Emerging 3D printing technologies for drug delivery devices: Current status and future perspective. *Advanced Drug Delivery Reviews*. 2021, 174. doi: 10.1016/j.addr.2021.04.019.

[71] CLOHESSY, R., COHEN, D., STUMBRAITE, K., BOYAN, B. and SCHWARTZ, Z. In vivo evaluation of an electrospun and 3D printed cellular delivery device for dermal wound healing. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2020, 108. doi: 10.1002/jbm.b.34587.

[72] BELLEGHEM, S. V., TORRES, L., SANTORO, M., MAHADIK, B., WOLFAND, A., KOFINAS, P. and FISHER, J. Hybrid 3D Printing of Synthetic and Cell-Laden Bioinks for Shape Retaining Soft Tissue Grafts. *Advanced Functional Materials*. 2019, 30. doi: 10.1002/adfm.201907145.

[73] MANDRYCKY, C., WANG, Z., KIM, K. and KIM, D.-H. 3D bioprinting for engineering complex tissues. *Biotechnology Advances*. 2016, 34, pp. 422–434. doi: 10.1016/j.biotechadv.2015.12.011.

[74] CAUSA, F., NETTI, P. and AMBROSIO, L. A multi-functional scaffold for tissue regeneration: The need to engineer a tissue analogue. *Biomaterials*. 2007, 28, pp. 5093–5099. doi: 10.1016/j.biomaterials.2007.07.030.

[75] LAROCHELLE, E., STREETER, S., LITTLER, E. and RUIZ, A. 3D-Printed Tumor Phantoms for Assessment of In Vivo Fluorescence Imaging Analysis

Methods. *Molecular Imaging and Biology*. 2023, 25, pp. 212–220. doi: 10.1007/s11307-022-01783-5.

[76] JIANG, Z., DIGGLE, B., TAN, M., VIKTOROVA, J., BENNET, C. and CONNAL, L. Extrusion 3D Printing of Polymeric Materials with Advanced Properties. *Advanced Science*. 2020, 7. doi: 10.1002/advs.202001379.

[77] GANTUMUR, E., KIMURA, M., TAYA, M., HORIE, M., NAKAMURA, M. and SAKAI, S. Inkjet micropatterning through horseradish peroxidase-mediated hydrogelation for controlled cell immobilization and microtissue fabrication. *Biofabrication*. 2020, 12. doi: 10.1088/1758-5090/ab3b3c.

[78] WANG, W., ZHU, X., LI, L., QIAN, B. and XIE, Y. Characterization of thermal inkjet droplets jitter. *Journal of Physics D: Applied Physics*. 2019, 52. doi: 10.1088/1361-6463/ab1e33.

[79] XU, C., ZHENG, M., HUANG, Y., OGALE, A., FU, J. and MARKWALD, R. Study of droplet formation process during drop-on-demand inkjetting of living cell-laden bioink. *Langmuir*. 2014, 30, pp. 9130–9138. doi: 10.1021/la501430x.

[80] HÖLZL, K., LIN, S., TYTGAT, L., VLIERBERGHE, S. V., GU, L. and OVSIANIKOV, A. Bioink properties before, during and after 3D bioprinting. *Biofabrication*. 2016, 8, pp. 032002–032020. doi: 10.1088/1758-5090/8/3/032002.

[81] OZBOLAT, I. and HOSPODIUK, M. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials*. 2016, 76, pp. 321–343. doi: 10.1016/j.biomaterials.2015.10.076.

[82] GUILLOTIN, B. et al. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials*. 2010, 31, pp. 7250–7256. doi: 10.1016/j.biomaterials.2010.05.055.

[83] GUILLEMOT, F. et al. High-throughput laser printing of cells and biomaterials for tissue engineering. *Acta Biomaterialia*. 2010, 6, pp. 2494–2500. doi: 10.1016/j.actbio.2009.09.029.

[84] JANG, T.-S., JUNG, H.-D., PAN, H., HAN, W., CHEN, S. and SONG, J. 3D printing of hydrogel composite systems: Recent advances in technology for tissue engineering. *International Journal of Bioprinting*. 2018, 4. doi: 10.18063/ijb.v4i1.126.

[85] LEIJTEN, J. et al. Spatially and temporally controlled hydrogels for tissue engineering. *Materials Science and Engineering: R: Reports*. 2017, 119, pp. 1–35. doi: 10.1016/j.mser.2017.07.001.

[86] SMITHMYER, M., SAWICKI, L. and KLOXIN, A. Hydrogel scaffolds as in vitro models to study fibroblast activation in wound healing and disease. *Biomaterials Science*. 2014, 2, pp. 634–650. doi: 10.1039/C3BM60319A.

[87] MURPHY, S. and ATALA, A. 3D bioprinting of tissues and organs. *Nature Biotechnology*. 2014, 32, pp. 773–785. doi: 10.1038/nbt.2958.

[88] PETTA, D., ARMIENTO, A., GRIJPMA, D., ALINI, M., EGLIN, D. and D'ESTE, M. 3D bioprinting of a hyaluronan bioink through enzymatic-and visible light-crosslinking. *Biofabrication*. 2018, 10, pp. 044104–044114. doi: 10.1088/1758-5090/aadf58.

[89] DOBOS, A. et al. Thiol–Gelatin–Norbornene Bioink for Laser-Based High-Definition Bioprinting. *Advanced Healthcare Materials*. 2019, pp. 1900752–1900761. doi: 10.1002/adhm.201900752.

[90] SINGH, Y., BANDYOPADHYAY, A. and MANDAL, B. 3D Bioprinting Using Cross-Linker-Free Silk–Gelatin Bioink for Cartilage Tissue Engineering. *ACS Applied Materials Interfaces*. 2019, 11, pp. 33684–33696. doi: 10.1021/acsami.9b11644.

[91] OUYANG, L., YAO, R., ZHAO, Y. and SUN, W. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*. 2016, 8, pp. 035020–035033. doi: 10.1088/1758-5090/8/3/035020.

[92] LI, Z., HUANG, S., LIU, Y., YAO, B., HU, T., SHI, H., XIE, J. and FU, X. Tuning Alginate-Gelatin Bioink Properties by Varying Solvent and Their Impact on Stem Cell Behavior. *Scientific Reports*. 2018, 23, pp. 8020–8028. doi: 10.1038/s41598-018-26407-3.

[93] POTTER, C., LAO, K., ZENG, L. and XU, Q. Role of Biomechanical Forces in Stem Cell Vascular Lineage Differentiation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2014, 34, pp. 2184–2190. doi: 10.1161/ATVBAHA.114.303423.

[94] BLAESER, A., CAMPOS, D. D., PUSTER, U., RICHTERING, W., STEVENS, M. and FISCHER, H. Controlling Shear Stress in 3D Bioprinting is a Key Factor to Balance Printing Resolution and Stem Cell Integrity. *Advanced Healthcare Materials*. 2016, 5, pp. 326–333. doi: 10.1002/adhm.201500677.

[95] URUSHIBARA, A., KODAMA, S. and YOKOYA, A. Induction of genetic instability by transfer of a UV-A-irradiated chromosome. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. 2014, 766. doi: 10.1016/j.mrgentox.2014.02.005.

[96] SCHWAB, A., LEVATO, R., D'ESTE, M., PILUSO, S., EGLIN, D. and MALDA, J. Printability and Shape Fidelity of Bioinks in 3D Bioprinting. *Chemical Reviews*. 2020, 120, pp. 11028–11055. doi: 10.1021/acs.chemrev.0c00084.

[97] REINA-ROMO, E., MANDAL, S., AMORIM, P., BLOEMEN, V., FERRARIS, E. and GERIS, L. Towards the Experimentally-Informed In Silico Nozzle Design Optimization for Extrusion-Based Bioprinting of Shear-Thinning Hydrogels. *Frontiers in Bioengineering and Biotechnology*. 2021, 9. doi: 10.3389/fbioe.2021.701778.

[98] HAYES, J., CURLEY, C., TIERNEY, P. and KENNEDY, J. Biomechanical analysis of a salt-modified polyvinyl alcohol hydrogel for knee meniscus applications, including comparison with human donor samples. *Journal of Mechanical Behavior of Medical Materials*. 2016, 56. doi: 10.1016/j.jmbbm.2015.11.011.

[99] GUO, L., COLBY, R., LUSIGNAN, C. and HOWE, A. Physical Gelation of Gelatin Studied with Rheo-Optics. *Macromolecules*. 2003, 36, pp. 10009–10020. doi: 10.1021/ma034266c.

[100] GHEBREMEDHIN, M., SEIFFERT, S. and VILGIS, T. Physics of agarose fluid gels: Rheological properties and microstructure. *Current Research in Food Science*. 2021, 4, pp. 436–448. doi: 10.1016/j.crfs.2021.06.003.

[101] JEJURIKAR, A., LAWRIE, G., MARTIN, D. and GRØNDAHL, L. A novel strategy for preparing mechanically robust ionically cross-linked alginate hydrogels. *Biomedical Materials*. 2011, 6. doi: 10.1088/1748-6041/6/2/025010.

[102] DUTTA, S., HEXIU, J., PATEL, D., GANGULY, K. and LIM, K. 3D-printed bioactive and biodegradable hydrogel scaffolds of alginate/gelatin/cellulose nanocrystals for tissue engineering. *International Journal of Biological Macromolecules*. 2021, 167, pp. 644–658. doi: 10.1016/j.ijbiomac.2020.12.011.

[103] SULTAN, S. and MATHEW, A. 3D printed scaffolds with gradient porosity based on a cellulose nanocrystal hydrogel. *Nanoscale*. 2018, 10, pp. 4421–4431. doi: 10.1039/C7NR08966J.

[104] ROH, H., KIM, H., KIM, C. and LEE, K. 3D Printing of Polysaccharide-Based Self-Healing Hydrogel Reinforced with Alginate for Secondary Cross-Linking. *Biomedicines*. 2021, 9. doi: 10.3390/biomedicines9091224.

[105] AHMAD, R., NAWAWI, W. W. and NASARUDDIN, R. Alginate and alginate composites for biomedical applications. *Asian Journal of Pharmaceutical Sciences*. 2021, 16, pp. 280–306. doi: 10.1016/j.ajps.2020.10.001.

[106] DAS, M., PARATHODIKA, A., MAJI, P. and NASKAR, K. Dynamic chemistry: The next generation platform for various elastomers and their mechanical properties with self-healing performance. *European Polymer Journal*. 2023, 186. doi: 10.1016/j.eurpolymj.2023.111844.

[107] MORGAN, F., MORONI, L. and BAKER, M. Dynamic Bioinks to Advance Bioprinting. *Advanced Healthcare Materials*. 2020, 9. doi: 10.1002/adhm.201901798.

[108] BATTA-MPOUMA, J., KANDHOLA, G., SAKON, J. and KIM, J. Covalent Crosslinking of Colloidal Cellulose Nanocrystals for Multifunctional Nanostructured Hydrogels with Tunable Physicochemical Properties. *Biomacromolecules*. 2022, 23, pp. 4085–4096. doi: 10.1021/acs.biomac.2c00417.

[109] HOEMANN, C., CHENITE, A., SUN, J., HURTIG, M., SERREQI, A., LU, Z., ROSSOMACHA, E. and BUSCHMANN, M. Cytocompatible gel formation of

chitosan-glycerol phosphate solutions supplemented with hydroxyl ethyl cellulose is due to the presence of glyoxal. *Journal of Biomedical Materials Research Part A*. 2007, 83, pp. 521–529. doi: 10.1002/jbm.a.31365.

[110] SHANGARI, N. and O'BRIEN, P. The cytotoxic mechanism of glyoxal involves oxidative stress. *Biomedical Pharmacology*. 2004, 68, pp. 1433–1442. doi: 10.1016/j.bcp.2004.06.013.

[111] CLAIR, M., BERMUDEZ, E., GROSS, E., BUTTERWORTH, B., RECIO, L. and CARRANO, A. Evaluation of the genotoxic potential of glutaraldehyde. *Environmental and Molecular Mutagenesis*. 1991, 18, pp. 113–119. doi: 10.1002/em.2850180206.

[112] KRISTIANSEN, K., POTTHAST, A. and CHRISTENSEN, B. Periodate oxidation of polysaccharides for modification of chemical and physical properties. *Carbohydrate Research*. 2010, 345, pp. 1264–1271. doi: 10.1016/j.carres.2010.02.011.

[113] UMAN, S., DHAND, A. and BURDICK, J. Recent advances in shear-thinning and self-healing hydrogels for biomedical applications. *Journal of Applied Polymer Science*. 2020, 137. doi: 10.1002/app.48668.

[114] KIM, S., KIM, D., ROH, H., KIM, H., LEE, J. and LEE, K. Three-Dimensional Bioprinting of Cell-Laden Constructs Using Polysaccharide-Based Self-Healing Hydrogelsg. *Biomacromolecules*. 2019, 20, pp. 1860–1866. doi: 10.1021/acs.biomac.8b01589.

[115] LI, S., PEI, M., WAN, T., YANG, H., GU, S., TAO, Y., LIU, X., ZHOU, Y., XU, W. and XIAO, P. Self-healing hyaluronic acid hydrogels based on dynamic Schiff base linkages as biomaterials. *Cabrohydrate Polymers*. 2020, 250. doi: 10.1016/j.carbpol.2020.116922.

[116] BILLMAN, J. and DIESING, A. Reduction of Schiff Bases with Sodium Borohydride. *The Journal of Organic Chemistry*. 1957, 22. doi: 10.1021/jo01360a019.

[117] BULCKE, A. V. D., BOGDANOV, B., ROOZE, N. D., SCHACHT, E., CORNELISSEN, M. and BERGHAMS, H. Structural and Rheological Properties of

Methacrylamide Modified Gelatin Hydrogels. *Biomacromolecules*. 2000, 1, pp. 31–38. doi: 10.1021/bm990017d.

[118] LUO, X., GUO, Z., HE, P., CHEN, T., LI, L., DING, S. and LI, H. Study on structure, mechanical property and cell cytocompatibility of electrospun collagen nanofibers crosslinked by common agents. *International Journal of Biological Macromolecules*. 2018, 113. doi: 10.1016/j.ijbiomac.2018.01.179.

[119] D'ESTE, M., EGLIN, D. and ALINI, M. A systematic analysis of DMTMM vs EDC/NHS for ligation of amines to hyaluronan in water. *Carbohydrte Polymers*. 2014, 108, pp. 239–246. doi: 10.1016/j.carbpol.2014.02.070.

[120] MUSILOVÁ, L., MRÁČEK, A., KOVALCIK, A., SMOLKA, P., MINAŘÍK, A., HUMPOLÍČEK, P., VÍCHA, R. and PONÍŽIL, P. Hyaluronan hydrogels modified by glycinated Kraft lignin: Morphology, swelling, viscoelastic properties and biocompatibility. *Carbohydrate Polymers*. 2018, 181, pp. 394–403. doi: 10.1016/j.carbpol.2017.10.048.

[121] PIEPER, J., WACHEM, P., LUYN, M., BROUWER, L., HAFMANS, T., VEERKAMP, J. and KUPPEVELT, T. Attachment of glycosaminoglycans to collagenous matrices modulates the tissue response in rats. *Biomaterials*. 2000, 21, pp. 1689–1699. doi: 10.1016/S0142-9612(00)00052-1.

[122] CAI, S., LIU, Y., ZHENG, S. and PRESTWICH, G. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials*. 2005, 26, pp. 6054–6067. doi: 10.1016/j.biomaterials.2005.03.012.

[123] HORN, E., BEAUMONT, M., SHU, X., HARVEY, A., PRESTWICH, G., HORN, K., GIBSON, A., PREUL, M. and PANITCH, A. Influence of cross-linked hyaluronic acid hydrogels on neurite outgrowth and recovery from spinal cord injury. *Journal of Neurosurgery: Spine*. 2007, 6, pp. 133–140. doi: 10.3171/spi.2007.6.2.133.

[124] GARCÍA-ASTRAIN, C., GANDINI, A., PEÑA, C., ALGAR, I., ECEIZA, A., CORCUERA, M. and GABILONDO, N. Diels–Alder "click" chemistry for the cross-linking of furfuryl-gelatin-polyetheramine hydrogels. *RSC Advances*. 2014, 4, pp. 2046–2069. doi: 10.1039/C4RA06122E.

[125] MADL, C. and HEILSHORN, S. Rapid Diels–Alder Cross-linking of Cell Encapsulating Hydrogels. *Chemistry of Materials*. 2019, 31, pp. 8035–8043. doi: 10.1021/acs.chemmater.9b02485.

[126] PIRMORADIAN, M., HOOSHMAND, T., NAJAFI, F., HAGHBIN, N. and DAVAIE, S. Design, synthesis, and characterization of a novel dual cross-linked gelatin-based bioadhesive for hard and soft tissues adhesion capability. *Biomedical Materials*. 2022, 17. doi: 10.1088/1748-605X.

[127] SPIVAK, A., DZENIS, Y. and RENEKER, D. A model of steady state jet in the electrospinning process. *Mechanics Research Communications*. 2000, 27, pp. 37–42. doi: 10.1016/S0093-6413(00)00060-4.

[128] MAZOOCHI, T., HAMADANIAN, M., AHMADI, M. and JABBARI, V. Investigation on the morphological characteristics of nanofiberous membrane as electrospun in the different processing parameters. *International Journal of Industrial Chemistry*. 2012, 3. doi: 10.1186/2228-5547-3-2.

[129] JAWOREK, A. and SOBCZYK, A. Electrospraying route to nanotechnology: An overview. *Journal of Electrostatics*. 2008, 66, pp. 197–219. doi: 10.1016/j.elstat.2007.10.001.

[130] FRAGKOPOULOS, A., ELLIS, P. and FERNANDEZ-NIEVES, A. Teaching Rayleigh–Plateau instabilities in the laboratory. *European Journal of Physics*. 2015, 36. doi: 10.1088/0143-0807/36/5/055023.

[131] RENEKER, D. and YARIN, A. Electrospinning jets and polymer nanofibers. *Polymer*. 2008, 49, pp. 2387–2425. doi: 10.1016/j.polymer.2008.02.002.

[132] BROWN, T., DALTON, P. and HUTMACHER, D. Direct Writing By Way of Melt Electrospinning. *Advanced Materials*. 2011, 23, pp. 5651–5658. doi: 10.1002/adma.201103482.

[133] PALANGETIC, L., REDDY, N., SRINIVASAN, S., COHEN, R. E., MCKINLEY, G. H. and CLASEN, C. Dispersity and spinnability: Why highly polydisperse polymer solutions are desirable for electrospinning. *Polymer*. 2014, 55, pp. 4920–4931. doi: 10.1016/j.polymer.2014.07.047.

[134] MALKIN, A., SEMAKOV, A., SKVORTSOV, I., ZATONSKIKH, P., KULICHIKHIN, V., SUBBOTIN, A. and SEMENOV, A. Spinnability of Dilute Polymer Solutions. *Macromolecules*. 2017, 50, pp. 8231–8244. doi: 10.1021/acs.macromol.7b00687.

[135] SHENOY, S., BATES, W., FRISH, H. and WNEK, G. Role of chain entanglements on fiber formation during electrospinning of polymer solutions: good solvent, no-specific polymer-polymer interaction limit. *Polymer*. 2005, 46, pp. 3372–3384. doi: 10.1016/j.polymer.2005.03.011.

[136] LIU, T., LIU, L., DING, F. and LI, Y. A Machine Learning Study of Polymer-Solvent Interactions. *Chinese Journal of Polymer Science*. 2022, 40, pp. 834–842. doi: 10.1007/s10118-022-2716-2.

[137] DEITZEL, J., KLEINMEYER, J., HARRIS, D. and TAN, N. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*. 2001, 42, pp. 261–272. doi: 10.1016/S0032-3861(00)00250-0.

[138] KUTALKOVA, E., INGR, M., KOLAŘÍKOVÁ, A., HRNČIŘÍK, J., WITASEK, R., HERMANOVÁ, M., ŠTRYMPL, O. and HUERTA-ÁNGELES, G. Structure and dynamics of the hyaluronan oligosaccharides and their solvation shell in water: organic mixed solvents. *Carbohydrate Polymers*. 2023, 304. doi: 10.1016/j.carbpol.2022.120506.

[139] KOLAŘÍKOVÁ, A., KUTÁLKOVÁ, E., BUŠ, V., WITASEK, R., HRNČIŘÍK, J. and INGR, M. Salt-dependent intermolecular interactions of hyaluronan molecules mediate the formation of temporary duplex structures. *Carbohydrate Polymers*. 2022, 286. doi: 10.1016/j.carbpol.2022.119288.

[140] ANGAMANNA, C. and JAYARAM, S. Investigation of the Optimum Electric Field for a Stable Electrospinning Process. *IEEE Transactions on Industry Applications*. 2012, 48, pp. 808–815. doi: 10.1109/TIA.2011.2180010.

[141] DEMIR, M., YILGOR, I., YILGOR, E. and ERMAN, B. Electrospinning of polyurethane fibers. *Polymer*. 2002, 43, pp. 3303–3309. doi: 10.1016/S0032-3861(02)00136-2.

[142] GHELICH, R., RAD, M. K. and YOUZBASHI, A. Study on Morphology and Size Distribution of Electrospun NiO-GDC Composite Nanofibers. *Journal of Engineered Fibers and Fabrics*. 2015, 10. doi: 10.1177/155892501501000102.

[143] STACHEWICZ, U., STONE, C., WILLIS, C. and BARBER, A. Charge assisted tailoring of chemical functionality at electrospun nanofiber surfaces. *Journal of Materials Chemistry*. 2012, 22. doi: 10.1039/C2JM33807F.

[144] JIRKOVEC, R., KALOUS, T. and CHVOJKA, J. The modification of the wetting of polycaprolactone nanofibre layers via alternating current spinning. *Materials & Design*. 2021, 210. doi: 10.1016/j.matdes.2021.110096.

[145] TONG, H. and WANG, M. Negative Voltage Electrospinning and Positive Voltage Electrospinning of Tissue Engineering Scaffolds: A Comparative Study and Charge Retention on Scaffolds. *Nano LIFE*. 2012, 2. doi: 10.1142/S1793984411000384.

[146] SIVAN, M., MADHESWARAN, D., HAUZEROVA, S., NOVOTNY, V., HEDVICAKOVA, V., JENCOVA, V., KOSTAKOVA, E., SCHINDLER, M. and LUKAS, D. AC electrospinning: impact of high voltage and solvent on the electrospinnability and productivity of polycaprolactone electrospun nanofibrous scaffolds. *Materials Today Chemistry*. 2022, 26. doi: 10.1016/j.mtchem.2022.101025.

[147] SZEWCZYK, P. and STACHEWICZ, U. The impact of relative humidity on electrospun polymer fibers: From structural changes to fiber morphology. *Advances in Colloid and Interface Science*. 2020, 286. doi: 10.1016/j.cis.2020.102315.

[148] ICOGLU, H. and OGULATA, R. Effect of ambient parameters on morphology of electrospun polyetherimide (PEI) fibers. *Tekstil ve Konfekslyon*. 2013, 23.

[149] HARIDAS, A., SHARMA, C., SRITHARAN, V. and RAO, T. Fabrication and surface functionalization of electrospun polystyrene submicron fibers with controllable surface roughness. *RSC Advances*. 2014, 4. doi: 10.1039/C3RA44170A.

[150] MARINO, T., RUSSO, F. and FIGOLI, A. The Formation of Polyvinylidene Fluoride Membranes with Tailored Properties via Vapour/Non-Solvent Induced Phase Separation. *Membranes*. 2018, 8. doi: 10.3390/membranes8030071.

[151] LI, D. et al. Fabrication and Applications of Multi-Fluidic Electrospinning Multi-Structure Hollow and Core–Shell Nanofibers. *Engineering*. 2022, 13, pp. 116–127. doi: 10.1016/j.eng.2021.02.025.

[152] ZHOU, Z., WU, X., DING, Y., YU, M., ZHAO, Y., JIANG, L., XUAN, C. and SUN, C. Needleless Emulsion Electrospinning for Scalable Fabrication of Core-Shell Nanofibers. *Journal of Applied Polymer Science*. 2014, 131. doi: 10.1002/APP.40896.

[153] SCHELSKI, K., REYES, C., PSCHYKLENK, L., KAUL, P. and LAGERWALL, J. Quantitative volatile organic compound sensing with liquid crystal core fibers. *Cell Reports Physical Sciences*. 2021, 2. doi: 10.1016/j.xcrp.2021.100661.

[154] VATS, S., ANYFANTAKIS, M., HONAKER, L., BASOLI, F. and LAGERWALL, J. Stable Electrospinning of Core-Functionalized Coaxial Fibers Enabled by the Minimum-Energy Interface Given by Partial Core-Sheath Miscibility. *Langmuir*. 2021, 37, pp. 13265–13277. doi: 10.1021/acs.langmuir.1c01824.

[155] MA, H., CHEN, G., ZHANG, J., LIU, Y., NIE, J. and MA, G. Facile fabrication of core-shell polyelectrolyte complexes nanofibers based on electric field induced phase separation. *Polymer*. 2017, 110, pp. 80–86. doi: 10.1016/j.polymer.2016.12.062.

[156] DEDE, S., SADAK, O., DIDIN, M. and GUNASEKARAN, S. Basil oil-loaded electrospun biofibers: Edible food packaging material. *Journal of Food Engineering*. 2022, 319. doi: 10.1016/j.jfoodeng.2021.110914.

[157] MA, J. et al. Enhanced viability of probiotics encapsulated within synthetic/natural biopolymers by the addition of gum arabic via electrohydrodynamic processing. *Food Chemistry*. 2023, 413. doi: 10.1016/j.foodchem.2023.135680.

[158] JAYAPRAKASH, P., MAUDHUIT, A., GAIANI, C. and DESOBRY, S. Encapsulation of bioactive compounds using competitive emerging techniques: Electrospraying, nano spray drying, and electrostatic spray drying. *Journal of Food Engineering*. 2023, 339. doi: 10.1016/j.jfoodeng.2022.111260.

[159] THOMPSON, C., CHASE, G., YARIN, A. and RENEKER, D. Effects of parameters on nanofiber diameter determined from electrospinning model. *Polymer*. 2007, 48, pp. 6913–6922. doi: 10.1016/j.polymer.2007.09.017.

[160] SUBRAHMANYA, T. M., ARSHAD, A., LIN, P., WIDAKDO, J., H.K. MAKARI, H. A., HU, C., LAI, J. and HUNG, W. A review of recent progress in polymeric electrospun nanofiber membranes in addressing safe water global issues. *RSC Advances*. 2021, 11, pp. 9638–9663. doi: 10.1039/D1RA00060H.

[161] PAPAPARASKEVA, G., PAPAGIORGIS, P., ITSKOS, G. and KRASIA-CHRISTOFOROU, T. Highly Aligned Electrospun Polymer Fibers Produced Using a Corrugated Static Collector. *ACS Applied Polymer Materials*. 2022, 4, pp. 586–597. doi: 10.1021/acsapm.1c01435.

[162] BUSKERMOLEN, A., SURESH, H., SHISHVAN, S., VIGLIOTTI, A., DESIMONE, A., KURNIAWAN, N., BOUTEN, C. and DESHPANDE, V. Entropic Forces Drive Cellular Contact Guidance. *Biophysical Journal*. 2019, 116, pp. 1994–2008. doi: 10.1016/j.bpj.2019.04.003.

[163] CHOWDHURY, B. M. F., CHARI, D. and ADAMS, C. Systematic Alignment Analysis of Neural Transplant Cells in Electrospun Nanofibre Scaffolds. *Materials*. 2023, 16. doi: 10.3390/ma16010124.

[164] PRABHAKARAN, M., VENUGOPAL, J. and RAMAKRISHNA, S. Mesenchymal stem cell differentiation to neuronal cells on electrospun nanofibrous substrates for nerve tissue engineering. *Biomaterials*. 2009, 30. doi: 10.1016/j.biomaterials.2009.05.057.

[165] WARREN, P., DAVIS, Z. and FISHER, M. Parametric control of fiber morphology and tensile mechanics in scaffolds with high aspect ratio geometry produced via melt electrowriting for musculoskeletal soft tissue engineering.

ournal of the Mechanical Behavior of Biomedical Materials. 2019, 99, pp. 153–160. doi: 10.1016/j.jmbbm.2019.07.013.

[166] VÍTKOVÁ, L., MUSILOVÁ, L., ACHBERGEROVÁ, E., KOLAŘÍK, R., MRLÍK, M., KORPASOVÁ, K., MAHELOVÁ, L., CAPÁKOVÁ, Z. and MRÁČEK, A. Formulation of Magneto-Responsive Hydrogels from Dually Cross-Linked Polysaccharides: Synthesis, Tuning and Evaluation of Rheological Properties. *International Journal of Molecular Sciences*. 2022, 23. doi: 10.3390/ijms23179633.

[167] KOPECKÁ, K. et al. Synthesis and Exfoliation of Calcium Organophosphonates for Tailoring Rheological Properties of Sodium Alginate Solutions: A Path toward Polysaccharide-Based Bioink. *Biomacromolecules*. 2023. doi: 10.1021/acs.biomac.3c00081.

[168] VÍTKOVÁ, L., MUSILOVÁ, L., ACHBERGEROVÁ, E., MINAŘÍK, A., SMOLKA, P., WRZECIONKO, E. and MRÁČEK, A. Electrospinning of Hyaluronan Using Polymer Coelectrospinning and Intermediate Solvent. *Polymers*. 2019, 11. doi: 10.3390/polym14030391.

[169] ZHU, M., TAN, J., LIU, L., TIAN, J., LI, L., LUO, B., ZHOU, C. and LU, L. Construction of biomimetic artificial intervertebral disc scaffold *via* 3D printing and electrospinning. *Materials Science Engineering C*. 2021, 128. doi: 10.1016/j.msec.2021.112310.

[170] FARSI, M., ASEFNEJAD, A. and BAHARIFAR, H. A hyaluronic acid/PVA electrospun coating on 3D printed PLA scaffold for orthopedic application. *Progress in Biomaterials*. 2022, 11. doi: 10.1007/s40204-022-00180-z.

[171] FANG, Y., LIU, Z., WANG, H., LUO, X., XU, Y., CHAN, H., LV, S., TAO, Y. and LI, M. Implantable Sandwich-like Scaffold/Fiber Composite Spatiotemporally Releasing Combretastin A4 and Doxorubicin for Efficient Inhibition of Postoperative Tumor Recurrence. *Applied Materials Interfaces*. 2022, 14, pp. 27525–27537. doi: 10.1021/acsami.2c02103.

[172] CALEJO, I., LABRADOR-RACHED, J., GOMEZ-FLORIT, M., REIS, R., DOMINGUES, R. and GOMES, M. Bioengineered 3D Living Fibers as In Vitro

Human Tissue Models of Tendon Physiology and Pathology. *Advanced Healthcare Materials*. 2022, 11. doi: 10,1002/adhm.202102863.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Ca3.1	mixed calcium-phosphonate phosphate
CaPhP	calcium phenylphosphonate
CIP	carbonyl iron microparticle
DEX-OX	dextran polyaldehyde
DMTMM	4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride
DO	degree of oxidation
DS	degree of substitution
ECM	extracellular matrix
EDC	1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide
EtOH	ethanol
HA	hyaluronan
HA-ADH	adipic acid dihydrazide grafted hyaluronan
HA-OX	hyaluronan polyaldehyde
HOBt	1-hydroxybenzotriazole
M_w	molecular weight
MCP	multicore particle
MeOH	methanol
MRE	magneto-rheological effect
NaAlg	sodium alginate
NHS	N-hydroxysuccinimide
NP	nanoparticle
PCL	poly-ε-caprolactone
PEO	polyethylene oxide
PVA	polyvinyl alcohol
SLP	specific loss power

PUBLICATIONS OF THE AUTHOR

[P.1] I.S. Smolkova*, N.E. Kazantseva, **L. Vitkova**, V. Babayan, J. Vilcakova and P. Smolka *Size Dependent Heating Efficiency of Multicore Iron Oxide Particles in Low-Power Alternating Magnetic Fields*. Acta Physica Polonica A, 2017, 131(4). https://doi.org/10.12693/APhysPolA.131.663.

[P.2] **L. Vitkova**, L. Musilova, E. Achbergerova, A. Minarik, P. Smolka, E. Wrzecionko and A. Mracek* *Electrospinning of Hyaluronan Using Polymer Coelectrospinning and Intermediate Solvent*. Polymers, 2019, 11(1517). https://doi.org/10.3390/polym11091517.

[P.3] L. Musilova, E. Achbergerova, **L. Vitkova**, R. Kolarik, M. Martinkova, A. Minarik, A. Mracek*, P. Humpolicek and J. Pecha *Cross-Linked Gelatine by Modified Dextran as a Potential Bioink Prepared by a Simple and Non-Toxic Process*. Polymers, 2021, 1(391). https://doi.org/10.3390/polym14030391

[P.4] **L. Vitkova**, L. Musilova*, E. Achbergerova, R. Kolarik, M. Mrlik, K. Korpasova, L. Mahelova, Z. Capakova and A. Mracek* *Formulation of Magneto-Responsive Hydrogels from Dually Cross-Linked Polysaccharides: Synthesis, Tuning and Evaluation of Rheological Properties*. International Journal of Molecular Sciences, 2022, 23(9633). https://doi.org/10.3390/ ijms23179633

[P.5] M. Jurtik, B. Greskova, Z. Pruckova, M. Rouchal, L. Dastychova, **L. Vitkova**, K. Valaskova, E. Achbergerova and R. Vicha* *Assembling a supramolecular 3D network with tuneable mechanical properties using adamantylated cross-linking agents and β-cyclodextrin-modified hyaluronan*. Carbohydrate Polymers, 2023, 313. https://doi.org/10.1016/j.carbpol.2023.120872

[P.6] K. Kopecka, **L. Vitkova***, Z. Kronekova, L. Musilova, P. Smolka, F. Mikulka, K. Melanova, P. Knotek, M. Humenik, A. Minarik and A. Mracek* *Synthesis and Exfoliation of Calcium Organophosphonates for Tailoring Rheological Properties of Sodium Alginate Solutions: A Path to-

ward Polysaccharide-Based Bioink. Biomacromolecules, 2023, *Accepted for publication*. https://doi.org/10.1021/acs.biomac.3c00081

[P.7] **L. Vitkova**, I. Smolkova, N. Kazantseva, L. Musilova, P. Smolka*, K. Valaskova, K. Kocourkova, M. Humenik, A. Minarik, P. Humpolicek and A. Mracek *Magneto-responsive hyaluronan hydrogel for hyperthermia and bioprinting: magnetic, rheological properties and biocompatibility*. APL Bioengineering, *Under review*.

[P.8] S. Emebu*, R.O. Ogunleye, E. Achbergerová, **L. Vítková**, P. Ponížil, C. Menodza-Martinez* *Review and proposition for model-based multivariable-multiobjective optimisation of extrusion-based bioprinting*. Applied Materials Today, *Submitted*.

AUTHOR'S CV

EDUCATION

Tomas Bata University in Zlín
9/2019-Currently
Ph.D. studies in Technology of Macromolecules

Tomas Bata University in Zlín
9/2014 - 6/2019
Bachelor's and Master's degree in Materials Engineering

INTERNATIONAL EXPERIENCE

University of Luxembourg
9/2021-10/2021 (8 weeks)
An internship in the group of Experimental Soft Matter Physics led by prof. Jan Lagerwall.

University of Bayreuth
11/2022-12/2022 (3 weeks)
An internship at the Chair of Biomaterials led by prof. Thomas Scheibel. The internship was supervised by assoc. prof. Martin Humenik.

PROJECT PARTICIPATION

IGA/FT/2023/006: Study and design of new processing procedures of polymer-based materials for advanced additive manufacturing
Team member

IGA/FT/2022/009: Hyaluronic acid and protein-based materials for 3D printing from theoretical and applied chemistry perspectives
Lead investigator

IGA/FT/2021/010: Preparation of advanced biopolymer systems

Team member

JUNG-2020-007: Development of biopolymer-based hydrogels for microextrusion
Lead investigator

IGA/FT/2020/005: Preparation of structured materials based on biopolymers
Team member

IGA/FT/2019/012: Preparation of structured biomaterials
Team member

Development of polymer systems suitable for processing via advanced technologies of 3D printing and electrospinning

Vývoj polymerních systémů vhodných pro zpracování pomocí pokročilých technologií 3D tisku a elektrostatického zvlákňování

Doctoral Thesis Summary

Published by: Tomas Bata University in Zlín

nám. T. G. Masaryka 5555, 760 01 Zlín.

Edition: published electronically

Typesetting by: Lenka Vítková

This publication has not undergone any proofreading or editorial review.

Publication year: 2023

First Edition

ISBN 978-80-7678-182-5