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Microstructure and mechanical behavior of cross-linked biopolymer networks

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Summary

In this thesis the microstructure and the response of a discrete three-dimensional isotropic network of cross-linked and unbundled fibers is studied numerically. A periodic representative volume element (RVE) of a 3D random network of cross-linked fibers is self-assembled by simulating the dynamics of fibers immersed in a water environment that is governed by a weak $1/r^2$ attractive potential between fibers (§ 2.2). The mechanical response of the networks is then calculated by subjecting the RVEs to simple shear in a separate, quasi-static finite element simulation (§ 2.3). In the network model, individual fibres are represented discretely by elastic beam elements that can stretch, bend and twist (§ 2.1). The fiber interconnections by cross-links are modelled as linear springs (§ 2.4). By keeping the cross-link spring constants very stiff, the microstructure and mechanical behavior of rigidly cross-linked networks (RCL) is first investigated in Chapter 3, Chapter 4 and Chapter 5. The mechanics of networks with compliant cross-links is addressed in Chapter 6.

In Chapter 3 it is demonstrated that simple relationships between geometrical and topological parameters of isotropically cross-linked network can describe the microstructure to great detail in terms of a few measurable macroscopic parameters. By using mere topological considerations, analytical expressions have been derived between the key length scale of the microstructure, i.e. the mean distance between two neighbouring cross-links along the filament, l_c, the network constituent concentrations and the average filament length l_0. This marks a significant improvement over methods available to date, which relied on inverse modeling using an assumed theoretical model for the network behavior. In addition, it is found that l_c scales with the $-1/3$rd power of the cross-link concentration, which is in good agreement with the exponent -0.4 obtained for F-actin networks cross-

linked by heavy-meromyosin (HMM) protein in the rigor state or exponent -0.2 suggested for ionically cross-linked networks of intermediate filaments.

The RCL networks at small strains are non-affine, bending dominated structures because the mean cross-link coordination (≤ 4) is below the isostatic limit (coordination ≥ 6) and because the filament sections in between two cross-links are long and slender ($l_{\mathrm{b}} \ll l_{\mathrm{c}}$). The complete scaling of the initial stiffness G_0 of RCL networks is found to comprise two contributions: general scaling characteristic for bending structures and an additional dependence on topology, amongst which is the distribution of cross-link coordination. The topological contributions are captured by a power-law dependence on two dimensionless numbers, that are of geometrical and topological nature.

The expression for the mean distance between cross-links l_{c} (Chapter 3) together with the scaling relation for the small strain RCL network response (Chapter 4) allows for prediction of the initial elastic shear modulus G_0 for any RCL network just by knowing the macroscopic parameters: the concentration of filaments c_{f}, the concentration of cross-linking molecules c_{cp} and filament length l_0. When compared with the small strain behavior of experimentally studied F-actin/rigor-HMM networks, the scaling relation predicts rather well the initial shear modulus G_0 of networks with lower actin concentrations over the whole range of HMM concentrations.

With continued straining, the RCL network modulus remains more or less constant up to a critical strain Γ_{c} or stress T_{c}. Beyond this level, the stiffness increases in a nonlinear fashion and is characterized by a power law dependence on macroscopic stress as $G \propto \mathrm{T}^{3/2}$. By carrying out the appropriate normalization, i.e. $G/G_0 = \tilde{G}$, $\Gamma/\Gamma_{\mathrm{c}} = \tilde{\Gamma}$ and $\mathrm{T}/\mathrm{T}_{\mathrm{c}} = \tilde{\mathrm{T}}$, the strain or stress-dependent stiffness collapses on a master curve, indicating the universal nonlinear behavior of the networks. The master curves as well as the exponent $3/2$ are in good agreement with the experiments performed on various *in vitro* networks of rigidly cross-linked filaments.

Detailed analysis of the distorting microstructure during deformation has revealed that the nonlinear network response is dominated by almost fully extended percolating paths comprised of axially stressed sections, i.e. a so-called stress path. Thus, contrary to the linear network response, which originates from the collective reorientation and bending of all network sections (Chapter 4), strain stiffening is governed by a highly localized phenomena.

There is a clear similarity between undulations in network stress paths and thermally undulating filaments in that the tensile response of both diverges as the slack is pulled-out; it is this that allows interpretation of the large strain RCL network response in terms of single filament mechanics and what explains the $3/2$

exponent governing the large strain scaling of the stiffness. However, there is also a distinct physical difference. While thermal undulations arise from the filament interacting with its environment, the slack of the network stress paths emerges purely from the network microstructure.

It is shown that the critical strain Γ_c of RCL network is an intrinsic property of the network microstructure that depends only on the connectivity. The shift of Γ_c with increasing network connectivity towards smaller values qualitatively corresponds to the experimentally-observed trend that Γ_c decreases with increasing concentration of cross-linking proteins. This trend is expected, since the consequence of increasing the concentration of the cross-linking proteins is a higher network connectivity.

These results, presented in Chapter 3, Chapter 4 and Chapter 5, provide a detailed description of the mechanical behavior of rigidly cross-linked networks, thereby emphasizing the importance of the network microstructure for the mechanical behavior. The ways in which the microstructure influences the network response is found to be key in matching the experimentally observed trends, both qualitatively and quantitatively.

It is very interesting to note that despite the differences in microstructure and/or properties of the constituents, the nonlinear stiffening of a wide range of biopolymer networks shows quite similar trends. Therefore, in Chapter 6 the relationship is presented between the response of a discrete 3D random network of cross-linked fibres and the material properties of the fibers, the cross-links and the network microstructure. The observation that the nonlinear network response is dominated by the behavior of a localised stress path allows for a simple rationalization of the strain-stiffening behavior in terms of two fundamental mechanisms. The first of these is a bending-dominated mechanism that can be characterised by a power-law dependence with exponent 3/2 of the normalized shear modulus $\tilde{G}$ on normalized stress $\tilde{\mathrm{T}}$ and is relevant for, e.g. the RCL networks (Chapter 5). The second mechanism is stretching-dominated and is pronounced when the cross-links are relatively more compliant than the fiber sections, i.e. it is a finite strain effect induced by reorientation of the stress path and gives rise to a power law between $\tilde{G}(\tilde{\mathrm{T}})$ with exponent 1/2.

The relative importance of the two mechanisms in the stiffening response can be expressed through the characteristic ratio $\bar{l}_b/l_c$ in terms of the effective axial-to-bending stiffness parameter $\bar{l}_b$ of filaments and cross-links. It is shown that the effective characteristic ratio $\bar{l}_b/l_c$ is a parameter that controls the nonlinear strain-stiffening ability of a cross-linked network, both in determining the critical point for the onset of nonlinearity and the bending-to-stretching transition. Because $\bar{l}_b/l_c$ relates material properties of the network constituents (bending and axial stiffnesses) to the key length scale of the microstructure, the same network responses can be obtained in various ways for different networks. Therefore, a plot of the normalised shear modulus $\tilde{G}$ against normalised stress $\tilde{\mathrm{T}}$ for a specific $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}}$ is suggested to represent the master curve for the large strain behavior of all networks associated to a particular $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}}$.

Networks with very low characteristic ratio, $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}} \ll 1$, exhibit bending-dominated 3/2 power law stiffening. Examples of networks that stiffen in this way all the way up to fracture are F-actin/scruin networks and cross-linked neuro-filaments. Networks associated with a somewhat higher characteristic ratio, e.g. ≈ 0.01, tend to show the transition from 3/2 to 1/2 power law stiffening. However due to network fracture, it may be difficult to distinguish this transition between stiffening mechanisms from convergence towards the upper limit set by the finite effective stiffness of the stress path. For networks with $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}} \approx 0.1$, the large strain response is mainly dominated by the stretching mechanism, leading to stiffening with 1/2 power law exponent. An even larger characteristic ratio $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}}$ may arise from rather compliant filament inter-connections, e.g rigor-HMM cross-linking molecules in the case of F-actin or branching points in the case of branched collagen I type networks. For networks with $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}} \rightarrow 1$, the stiffening ability due to the 1/2 mechanism is greatly diminished while the onset of nonlinearity is being pushed to larger strains. Such trends can be observed in bundled F-actin/fascin networks at high fascin concentrations, where networks are found unable to stiffen at all. In conclusion, various biopolymer networks of different constituents and rather different microstructure show strain-stiffening trends that can all be understood in terms of the competition between two stiffening mechanisms.

Samenvatting

Dit proefschrift handelt over biofysische netwerken: drie-dimensionale structuren van lange biopolymere filamenten die met speciale, zogenaamde cross-link moleculen met elkaar verknoopt zijn. Hoewel de samenstelling van biofysische netwerken sterk verschilt, is hun biologische functie dezelfde: namelijk, om het weefsel of de cel mechanische stijfheid te verlenen. Net zoals een elastiekje hebben biofysische netwerken de neiging om stijver te worden naarmate zij verder worden vervormd; de oorsprong van deze versteviging is echter een heel andere dan in rubber. Een veelheid van experimenteel onderzoek gedurende de afgelopen twee decennia heeft veel gegevens opgeleverd over een breed palet van in-vitro samengestelde netwerken, maar omdat de theorievorming zich heeft toegespitst op een aantal klassen van netwerken is het begrip tot nu fragmentarisch en kwalitatief. In dit proefschrift wordt voor een brede klasse van biofysische netwerken een geünificeerde verklaring van hun mechanische eigenschappen gepresenteerd die de nadruk legt op de microstructuur van het netwerk.

Hiertoe worden numerieke simulaties uitgevoerd aan drie-dimensionale netwerken bestaande uit een discreet aantal, lange semiflexibele filamenten met een willekeurige oriëntatie in de ruimte die op een variërend aantal plaatsen aan elkaar verknoopt zijn door elementen die cross-links representeren. De mechanische eigenschappen van de filamenten en de cross-links zelf zijn lineair verondersteld en kunnen over een groot gebied gevarieerd worden teneinde een breed scala aan netwerken te kunnen bestuderen.

Een belangrijk limietgeval wordt gevormd door netwerken die verknoopt zijn door onvervormbare cross-links. Voor dit soort netwerken wordt aangetoond dat, onder fysiologisch relevante condities, de stijfheid bepaald wordt door de buigstijfheid van de filamenten en door slechts één karakteristieke eigenschap van de mi-

crostructuur, namelijk de verhouding tussen de gemiddelde filamentlengte en de gemiddelde afstand tussen cross-links langs de filamenten. Deze microstructuurparameter is niet ogenblikkelijk te meten, maar topologische beschouwingen staan ons in staat deze karakteristieke lengteschalen uit te drukken in meetbare grootheden, zoals de concentratie van de filamenten en van de cross-links.

In overeenstemming met experimentele waarnemingen blijft de stijfheid van de netwerken slechts tot zekere vervorming (of spanning) constant. Na een kritisch punt, blijkt de numeriek berekende stijfheid toe te nemen met de spanning tot de macht 3/2. Deze schalingswet wordt in de literatuur vaak gezien als een entropisch effect als gevolg van thermische undulaties in de filamenten, maar in hoofdstuk 5 wordt aangetoond dat het meer in het algemeen een gevolg is van het straktrekken van filamenten. Een aantal vrijwel strakgetrokken filamenten ontstaat geleidelijk door her-oriëntatie van filamenten tijdens de sterk niet-affiene vervorming in een discreet netwerk. De simulaties laten bovendien zien dat de kritische vervorming waarbij versteviging begint een intrinsieke eigenschap is van een netwerk die uitsluitend afhangt van diens mate van verknoping.

Nu bovengenoemde universele wetmatigheden zijn gevonden voor netwerken die star met elkaar verknoopt zijn, wordt de klasse van materialen uitgebreid door de vervormbaarheid van cross-links toe te staan. Ook dit soort netwerken kan versteviging vertonen, maar de wijze waarop hangt af van de verhouding tussen de stijfheid van de filamenten en die van de cross-links. Er blijken twee fundamentele mechanismen van versteviging te bestaan. De ene is degene die bij starre cross-links reeds naar voren is gekomen, de andere is een niet-lineair geometrisch effect dat optreedt bij reeds strakke filamenten en relatief flexibele cross-links. Daar waar het eerste mechanisme aanleiding geeft tot een schalingswet voor stijfheid als spanning met exponent 3/2, is de exponent voor het andere mechanisme slechts 1/2. Voorts wordt aangetoond dat de competitie tussen beide mechanismen bepaald wordt door de effectieve axiale stijfheid van filament plus cross-link en de gemiddelde afstand tussen cross-links. Uiteindelijk laat het palet van niet-lineair gedrag van biofysische netwerken, van diverse samenstelling en verschillende microstructuur, zich nu betrekkelijk eenvoudig samenvatten met deze ene experimenteel meetbare parameter.

Sažetak

Nasumična mreža povezanih vlakana ili niti je učestala mikrostruktura mnogih materijala, od metalnih pjena s otvorenom ćelijom, filca, papira i gume, pa sve do struktura bio-polimernih filamenata u organskom tkivu i staničnom citoskeletu. Dok je očito da mehanička svojstva ovakvih materijala ovise o svojstvima primarnih komponenata, tj. vlaknu i unakrsnim vezama preko kojih se prenosi sila između vlakana, od posebnog značaja kod velikih deformacija je utjecaj same mikrostrukture. Do današnjeg dana, mnogobrojni eksperimenti su pokazali da *in vitro* rekonstruirane bio-polimerne mreže prilikom velikih, nelinearnih, ali još uvijek elastičnih deformacija mogu povećati svoju krutost za čak tisuću puta. Za usporedbu, mali broj sintetičkih polimernih materijala, iako fenomenološki sličnih bio-polimernim mrežama, mogu tek udvostručiti krutost kod velikih deformacija.

Usprkos svom dosadašnjem istraživanju, mehanička svojstva bio-polimernih mreža još uvijek kriju mnogo nerazriješenih i kontroverznih zanimljivosti. Osnovna pretpostavka u ovoj disertaciji je da je većina tih zanimljivosti skrivena i ovisna o mikrostrukturi same mreže. Stoga su ovdje, numeričkim metodama istraživani fundamentalni utjecaji mikrostrukture na mehanički odziv diskretnih trodimenzionalnih mreža načinjenih od unakrsno povezanih bio-polimernih niti.

Reprezentativni volumenski element (RVE) mreže je generiran samoudruživanjem, na način da je uz pomoć računalnog programa simulirana dinamika polimernih niti u vodenom okruženju uzrokovana slabom privlačnom silom između niti (§ 2.2). Mehanički odziv mreže je izračunat pomoću računalne simulacije bazirane na metodi konačnih elemenata u kojoj je stanje makroskopske deformacije RVE-a jednostavno smicanje (§ 2.3). U modelu mreže, polimerne niti su diskretizirane upotrebom elastičnih grednih elemenata formuliranih na način da se deformacija svakog grednog elementa sastoji od istovremenog istezanja, savijanja i uvijanja

(§ 2.1). Unakrsne veze između mrežnih niti, međutim, modelirane su kao linearne elastične opruge kontrolirane jakosti (§ 2.4).

Različita geometrijska i topološka svojstva mreže ovisna su o tek nekoliko makroskopskih parametara (vidi poglavlje 3). Između ostalog, uzimajući u obzir samo geometriju i topologiju mreže izvedena je jednadžba koja povezuje temeljni parametar geometrije mreže, tzv. srednju udaljenost između dvije uzastopne unakrsne veze l_c, sa srednjom ukupnom duljinom niti l_0 i koncentracijama niti c_f i unakrsnih veza c_cp u mreži. U usporedbi sa do sada prisutnim metodama za izračunavanje duljine l_c, koje zahtijevaju pretpostavke o načinu na koji se mreža deformira kao i izmjerena elastična svojstva mreže, izvedena jednadžba predstavlja praktičan i značajan doprinos za razumjevanje mikrostrukture bio-polimernih mreža. Nadalje, pokazano je da je srednja udaljenost između uzastopnih veza razmjerna koncentraciji veza, $l_\mathrm{c} \propto c_\mathrm{cp}^{-1/3}$, što je u suglasnosti s postojećim eksperimentima. Naime, slična razmjernost, ali s eksponentom -0.4 pronađena je u slučaju mreža sastavljenih od aktinskih filamenata (engl. *actin filaments*) povezanih pasivnim HMM (engl. *heavy-meromyosin in a rigor state*) bjelančevinama, dok je razmjernost s eksponentom -0.2 utvrđena u slučaju mreža od intermedijarnih filamenata (engl. *intermediate filaments*) povezanih ionskom vezom.

U slučaju malih deformacija, mreža s potpuno krutim unakrsnim vezama (RCL mreža) se deformira na ne-afin (engl. *non-affine*) način. Drugim riječima, kod malih deformacija, dominantno za sve niti je lokalno stanje savijanja zbog kojeg se lokalni tenzor deformacije unutar mreže bitno razlikuje od makroskopske deformacije. Razmjernost koja opisuje krutost mreže kod malih deformacija, G_0, značajno ovisi o raspodjeli koordinacije unakrsnih veza (poglavlje 4), koja je jednoznačno definirana omjerom između srednje duljine niti i srednje udaljenosti izmedju dvije uzastopne veze, l_0/l_c (vidi poglavlje 3). U usporedbi s eksperimentalno izmjerenim vrijednostima za krutost, G_0, mreža aktinskih filamenata povezanih pasivnim HMM molekulama, predviđanja na temelju ovdje izvedenih jednadžbi su u odličnoj suglasnosti u slučaju manjih koncentracija niti c_f.

Kod velikih deformacija, odziv RCL mreže je karakteriziran konstantnom krutošću G_0 sve do kritične deformacije Γ_c ili kritičnog naprezanja T_c. Međutim, iznad kritične deformacije, krutost RCL mreže, G, postaje izrazito nelinearna funkcija deformacije, koja može biti karakterizirana razmjerom, $G \propto \mathrm{T}^{3/2}$, s obzirom na naprezanje T. Detaljnom analizom mikrostrukture kod velikih deformacija pokazano je da su nelinearnosti u odzivu RCL mreže usko povezane sa uspostavljanjem puteva kroz mrežu koji su sastavljeni od dijelova niti sa visokim vlačnim naprezanjem, tzv. naprezajući put (engl. *stress path*).

Uspoređujući rezultate brojnih eksperimenata, moguće je primijetiti da odzivi vrlo različitih mreža, tj. mreža načinjenih od različitih niti, unakrsnih veza i bitno drukčije mikrostrukture, pokazuju slične trendove. Ova opservacija je interesantna jer sugerira na postojanje općenitih mehanizama odgovornih za nelinearnost odziva mreže (vidi poglavlje 6). Činjenica da su različite nelinearnosti odziva uvijek povezane sa formiranjem naprezajućih puteva, omogućava interpretaciju općenitog odziva kod velikih deformacija kroz dva osnovna mehanizma. U prvom mehanizmu, svojstvenom za RCL mreže (vidi poglavlje 5), dominantna deformacija je izvijanje dijelova niti u naprezajućim putevima, dok je razmjer između krutosti G i naprezanja T okarakteriziran s eksponentom 3/2. Međutim, za drugi mehanizam (vidi poglavlje 6), dominantno stanje deformacije je istezanje naprezajućih puteva, te eksponent u razmjernosti između krutosti G i naprezanja T je 1/2. Relativni značaj ova dva mehanizma za odziv mreže moguće je izraziti pomoću bezdimenzijskog mrežnog broja, $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}}$, koji uzima u obzir relativnu krutost između niti i unakrsnih veza, $\bar{l}_{\mathrm{b}}$, kao i karakterističnu veličinu mikrostrukture mreže, l_{c}. Kroz mrežni broj $\bar{l}_{\mathrm{b}}/l_{\mathrm{c}}$ stoga, na jedinstven način moguće je objasniti nelinearnosti u odzivu velikog broja bio-polimernih mreža, načinjenih od različitih vrsta niti i unakrsnih veza, te sa bitno različitom mikrostrukturom.

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