HYDROGELS WITH ENHANCED MECHANICAL STRENGTH AND THEIR MEDICO-PHARMACEUTICAL APPLICATION

KONSTANTINA HARRASS1, GEORGE GEORIEV2

1DWI e.V. and Institute of Technical and Macromolecular Chemistry, RWTH Aachen University, Forckenbeckstr. 50, 52074 Aachen, Germany
2Department of Chemistry and Pharmacy, Laboratory Water-Soluble polymers, University of Sofia St. Kl. Ohridski, J. Bourchier Ave., 1164 Sofia, Bulgaria
*Corresponding author: E-mail: georgs@chem.uni.sofia.bg.

Abstract: The methods for the preparation of the three main types of the hydrogels with enhanced mechanical characteristics (double network, nanocomposite, copolymer or polymer blend) are described. The latter, together with the possibilities for their effective control, are compared and discussed. Medicopharmaceutical applications of collagen, collagen-noncollagenous protein and collagen-glucosaminoglucan hydrogels, reinforced with nanohydroxyapatite and/or β-tricalcium phosphate nanoparticles are discussed as an example for bone graft materials, the second most common transplantation tissue.

Keywords: Hydrogel, Mechanical strength, Strain-at-break, Compression and tensile modulus, Collagen, Noncollagenous protein, Glycosaminoglucon, Bone tissue, Bone graft, Extracellular matrix, Osteoconductivity, Osteoinductivity, Osteointegration, Osteogenic cell, Bone tissue vascularization.

INTRODUCTION

Hydrogels consist of swollen water polymer networks with a specific amount of crosslinks, which are originated by physical or chemical bondings. Due to chemical residues exhibiting a hydrophilic character within the polymer chains and also a relatively low density of cross-links, the polymer network shows a distinctive ability to swell in aqueous solutions. It retains namely its shape, but an extensively increasing volume can be reached at equilibrium condition.
A wide range of chemical compositions can be used for hydrogel preparations whereby the only necessity from precursors is required to comprise hydrophilic groups and, of course, chemically (or physically) reactive substituents capable for cross-linked connections between polymer chains. The synthesis route creating a specific hydrogel network is usually initiated by thermo- or photopolymerization as well as in some cases also by electron-beam and gamma irradiation. Furthermore, the development of the network structure can also be controlled by reaction parameters, e.g. pH value, temperature, monomer concentration, activators as well as cross-linking agents [1]. Therefore, it is possible to achieve a nearly tailor-made swelling behaviour of the hydrogel, whereby water contents up to more than 90 per cent can be obtained.

Because of this advantageous feature, hydrogel polymers offer considerable options in the scope of medical and pharmaceutical applications. Hydrogels are involved in therapeutic applications (e.g. tissue engineering and drug delivery [2–3]) and diagnostic devices [4–5]. They play a role in analytical and biotechnological systems as actuator/sensor materials [6–7] and in forms of thin (multi)layers as biomaterials with patternable characteristics [8–9].

A major important use of hydrogels is to apply them in drug delivery systems giving pharmaceutical agents during medical therapy [10]. The hydrogel can be produced in a physical form, like a capsule, coating or sheet, and fixed on an implant, catheter or wound bandage. Then, a specific drug amount is released directly where the healing effect of an organ or tissue is needed. The ability of hydrogel to work as a donor of drugs depends mainly on its diffusion coefficient but also on the presence of pores and the specific structure of the hydrophilic network. Therefore, the controlling of polymer chain length, polymer composition, and initiator concentration is mainly required to manage the density and degree of network crosslinking [1, 11].

A release of therapeutics in drug-delivery applications occurs often by environmentally responsive hydrogels. The conditions of the environment (e.g. temperature or pH value) affect a response of the hydrogel matrix to deliver drugs in a defined amount [12–13]. The use of hydrogels in drug reservoir system are potentially feasible for many medical and biological applications but also limited often by the necessity to overcome practical circumstances like appropriate gel viscosity or mechanical stability [14].

Another field of medical interest of biocompatible hydrogels is involved in engineering human-like organs or tissues. By a number of different strategies, patients own cells taken by biopsy can be integrated in a hydrogel scaffold that act as analogues to the natural matrices found in tissues [15]. The hydrogel structure provides a space in the regular human environment and delivers human cells to desired sites of malfunctioned tissue, suffered by diseases or accidents. In tissue engineering applications, hydrogels can be able to substitute skins, membranes, implants and partially organs.

Hydrogels can act as synthetic biomaterials with biocompatible properties to give the microenvironment, the so-called extracellular matrix (ECM), for the implementation of different kinds of human cells. The specific characteristic of the tailor-made hydrogel formed the ECM determines the appropriateness as a biofunctional component to interact
with the type of cell or tissue, e.g. hydrogels including cell-adhesive ligands offer binding sites to surfaces of biological matter [16]. A relative new approach in tissue engineering is an organ printing procedure where a computer-driven deposition of cells occurs to create cell-laden hydrogel structures. In this field of regenerative medicine, the mechanical strength of hydrogels is of great importance because of difficulties in their handling and withstanding applied forces [17]. In tissue-engineering applications there is always a need to control the chemical and also mechanical properties of hydrogel matrices so that encapsulated and embedded cells can have a response to the environmental substrate in order to ensure their survival and subsequently a new tissue formation [18–20].

Beside the appropriateness of hydrogels to ensure biocompatibility and polymer structures with high water contents inside which satisfying transport mechanisms of biological fluids can occur, a demand of good mechanical properties is also required [21]. For many medical applications like implanting artificial tissue for cartilage or bone, nerve matrix tissue, skeletal muscles, among others, the strength of hydrogels must be determined in ranges where obviously the convenience of a patient can be guaranteed. Unfortunately, in many cases hydrogels offer in many cases a rather weak mechanical behaviour. For example, for cartilage tissue, a Young’s modulus and compression stress-at-break of 13 MPa and 1.9–14.4 MPa [22–23], respectively, was found which cannot be met by numerous hydrogels. But some strategies exist for improving the strength of hydrogels to a higher level. An overview of special preparation approaches and reinforcement techniques selected and summarized from recent literature are presented in this work.

HYDROGEL PREPARATION APPROACHES

DOUBLE NETWORK HYDROGELS

Double networks (DNs) are interpenetrating networks with a great difference between the crosslinking density of their components, low-density single network (LDSN) and high density single network (HDSN). DN hydrogels were usually synthesized using a two-step network formation procedure. A first monomer immersed in a solution reacts to a polymer network by common polymerization procedures, whereby crosslinkers and initiators can also be involved. In a second step after subsequent treatment of the single network obtained, a further polymerization reaction with another monomer leads to the development of the DN hydrogel.

In works of Osada et al. [24–33] in each case the first network was prepared in an aqueous solution of 1 M 2-acrylamido-2-methylpropanesulfonic acid (AMPS) by UV free radical polymerization. The mixture consisted additionally 4 mol % N,N’-methylene bisacrylamide (MBAA) as crosslinking agent (high concentration) and 0.1 mol % 2-oxoglutaric acid as initiator. After a six-hours-polymerization, the obtained PAMPS single network (SN) hydrogel obtained was immersed for one day in an aqueous solution of 2 M acrylamide (AAm) and 0.1 mol % 2-oxoglutaric acid (for case of [24–26]) or 3 M N,N’-dimethyl acrylamide (DMAAm) and 0.1 mol % potassium persulfate (KPS) (for case of
Additionally, in all aforementioned cases 0.1 mol % MBAA (low concentration) was used as crosslinking agent. Finally, a second network was achieved which is placed parallel to the location of PAMPS single network. The two polymer structures are entangled with each other and together they build in their sum the DN hydrogel.

In a further step, the DN hydrogels obtained were subsequently immersed in an aqueous solution of 1 M AMPS and 0.1 mol % 2-oxoglutaric acid with or without 0.1 mol % MBAA. In the absence of MBAA, a DN with non-crosslinked linear polymer chains was generated and is therefore called a DN-L [26, 34]. The presence of the crosslinker led to the formation of a triple network (TN) [26, 34].

In Ref. [25, 27–28, 35], Bacterial Cellulose (BC) as SN was used. In case of BC/PDMAAm DN hydrogel, BC was immersed in an aqueous solution of 3 M DMAAm, containing 0.1 mol % potassium persulfate and 0.1 mol % MBAA. After staying for one day in order to receive equilibrium conditions, a thermopolymerization performed through six hours at 60 °C yield to the DN hydrogel. For the BC/Gelatine DN hydrogel, BC was put in an aqueous solution of 30 wt. % Gelatine (pH 7.0) for one week at 50 °C. Subsequently, BC was immersed in an aqueous solution of 1 M 1-ethyl-(dimethylaminopropyl) carbodiimide (EDC) for four days at ambient temperature in order to obtain a chemical crosslinking network and its hydrogel.

In order to prepare a BC/PAAm DN hydrogel, pure BC hydrogel was immersed for two days to reach equilibrium conditions in an aqueous solution of AAm with concentrations of 0–4 M, respectively, whereas the 4 M solution contains correspondingly 0–5 mol % MBAA, and 0.1 mol % KPS. A supplemental approach to prepare BC/PAAm DN hydrogel represents a simple subtraction of water by compression and the subsequent immersion in a solution of 4 M AAm, which comprises 2.5 mol % MBAA and 0.1 mol % KPS. In each case, the PAAm SN is polymerized in the presence of BC hydrogel for six hours at 60 °C.

An innovative attempt, developed to enhance the mechanical properties of hydrogels representing free-shaped DN, comprises physically cross-linked poly(vinyl alcohol) (PVA) as an “inner template” [36]. In the initial stage, a solution of PVA in concentration 10 wt. % was cast in diverse shapes (as bird/ fish/ knot), quenched for 24 hours at –40 °C, and subsequently, the cross-linked structure obtained was cleaned. In the second step, PVA hydrogels were immersed in a solution consisting of 1 M AMPS, 4 mol % MBAA and 0.1 or 0.6 mol % 2-oxoglutaric acid for three days. Throughout the time of swelling, PVA hydrogels were kept under argon atmosphere and at sealed conditions, respectively, in order to avoid drying. After photo polymerization for eight hours, a PVA/PAMPS DN hydrogel was obtained, which was further immersed in solution of 2 M AAm and 0.01 mol % 2-oxoglutaric acid for two days. Subsequently, the PAAm SN was synthesized in the presence of already formed PVA/PAMPS DN. The generated hydrogel material was designated as PVA/PAMPS/PAAm triple-network (PVA-DN) hydrogel.

Based on the approach of Osada et al., a novel kind of DN was synthesized by Ajiro et al. [38]. An aqueous solution of 2 M N-vinylacetamide (NVA), containing 1 mol % N,N′-5-oxanomonemethylenbis-N-vinylacetamide (5ON-bis-NVA) as crosslinking agent and 1 mol % 2,2′-azobis(2-methylpropionamidine) dihydrochloride (V–50) as initiator was polymerized for four hours at 37 °C. The NVA SN hydrogel prepared in this way was then
swelled in an aqueous solution of 2 M NVA, 1 mol% 5ON-bis-NVA and 1 mol% V–50 for one day at 4 °C. A second network of NVA was subsequently formed in the already existing NVA SN by a polymerization for six hours at 37 °C.

A poly(ethylene glycol)-diacrylate/poly(acrylic acid) (PEG-DA(X)/PAA[Y]) DN hydrogel was introduced from Myung et al. [39]. An aqueous solution of purified PEG-DA and 1 wt.% 2-hydroxy-2-methyl-propiophenone as initiator was exposed to UV light for ten minutes. The PEG-DA SN hydrogel obtained was then immersed then in an AA solution, containing 1 v/v-% 2-hydroxy-2-methyl-propiophenone and 1 v/v-% triethylene glycol dimethacrylate (TEGDMA) as crosslinking agent. After staying for one day in order to reach equilibrium conditions, a further photo polymerization was carried out for five minutes.

Jhang et al. [40] prepared a physical-chemical zwiterion-containing DN. For this case, linear poly(sulfobetaine methacrylate) (polySBMA) with molecular weight 169 kDa was dissolved in a solution of 2 M NaCl, 2.5 M SBMA as monomer, 2.6 mol% ammonium persulfate and 1.2 mol% metasulfite as initiator. Furthermore, MBAA as a crosslinking agent in concentrations of 16, 40, 81,122 or 162 mM was added. A free radical polymerization was performed where the linear polySBMA was interpenetrated within covalently crosslinked polySBMA hydrogel.

A new type of DN hydrogel composed from hyaluronan/N,N′-dimethylacrylamide (PHA/PDMAAm) was reported from the group of Weng and collaborators [41]. HA SN hydrogel was prepared by a UV polymerization of 2 wt.% glycidyl methacrylate (GMA) derived by HA solution in the presence of initiator 0.1 mol% 2-oxoglutaric acid for two hours. After this, PHA was put to swell in 1–5 mol/L solution of DMAAm as monomer, consisting 0.1 mol% 2-oxoglutaric acid and 0, 0.01, 0.05, 0.1, 0.5, 1 or 2 mol% MBAA as crosslinker. Upon equilibrium of the PHA hydrogel with the DMAAm monomer solutions were reached, a second net-work was formed by irradiation with UV light for two hours.

A poly(vinyl alcohol)/poly(ethylene glycol) (PVA/PEG) DN hydrogels prepared by several cycles of freezing and thawing were introduced by Zhang et al. [42]. Definitely an amount of PVA (DP = 1750 ± 50) was dissolved in ultra-pure water while stirring for six hours at 90 °C. The solution obtained was divided into four parts and a different amount of PEG (M_w = 7500 g/mol) was supplemented to each of them. The mixtures achieved were stirred for two hours and subsequently exposed to several cycles of freezing for eight hours at −20 °C and thawing for four hours at ambient temperature.

Poly-ether-urethane-poly(methyl methacrylate) interpenetrating polymer net-works (PEU-PMMA IPNs) were synthesized by Rakovsky et al. [43]. A mixture of MMA as monomer, 0.5 wt.% benzoyl peroxide (BPO) as initiator and 5 mol% di(ethylene glycol) divinyl ether (DEGDVE) as crosslinking agent was prepared and held under nitrogen for at least one hour. An amount of this composition was added then to a molten PEG (M_w = 1000, 1500 or 2000 g/mol) which reacted preliminary with isophorane diisocyanate (IPDI). After 20 hours polymerization time at 70 °C, PEU-PMMA gels were obtained.

Recently published, an approach for a kind of triple network gel was introduced by Nakajima et al. [44]. A linear polymer molecule (named molecular stent that derived from a device in cardiovascular medicine) is inserted in order to increase as a polyelectrolyte the swelling capability of the first network. The neutral first network gel was prepared by photo-
polymerization at 365 nm UV-light of 2-hydroxyethylacrylate (HEA) in the presence of a cross-linker (N,N'-methylenebis (acrylamide), MBAA) and photoinitiator (2-oxoglutaric acid). The molecular stent inside the first network was then synthesized by the photo-polymerization of 2-acrylamido-2-methylpropanesulfonic acid (AMPS). The second network inside the stent-containing single network gel was prepared by photo-polymerization of AAm in the presence of MBAA and 2-oxoglutaric acid. In order to remove the molecular stent after final DN preparation, an ionic surfactant typically sodium dodecyl sulphate (SDS) was used instead to support the swelling behaviour but can be washed out easily.

The group of Haque et al. reported the production of the bilayer structure from poly dodecyl glyceryl itaconate (PDGI)/PAAm DN hydrogel \[45\]. The DGI was anisotropically orientated in the PAAm network by applying high shear forces during preparation at temperatures of 55 °C. After subsequent homo-polymerized reaction under the presence of MBAA as crosslinker and Irgacure photoinitiator, a lamellar structure of PDGI became stable inside the PAAm matrix. Due to the parallel aligned structure, extensive light diffraction in the visible range through deflection of layers could be observed during swelling as well as mechanical-stimulated strain.

Shin et al. presented a study about DN hydrogels based on gelatin and gellan gum macromolecules modified by methacrylate in alkaline solution and crosslinked through photo-polymerization under wavelength of 350–500 nm \[46\]. The gellan gum methacrylate (GGMA) creates the rigid and brittle first network while in a second step gelatin methacrylamide (GelMA) completes as a soft and ductile structure the biocompatible DN hydrogel.

In Table 1, the different kinds of DN hydrogels and each of their equilibrium water content (EWC) are listed. For Ref. \[39\], the EWC varied for the PEG-DA(X)/PAA[Y] DN hydrogel depending on pH value and molecular weight of PEG-DA. In Ref. \[41\], the EWC values correlate with the concentration of DAAm and MBAA, while in Ref. \[43\], the molecular weight of PEG, crosslinking density of IPDI as well as volume fraction of MMA were altered.

The EWC was determined in Table 1 by applying the relationship given in Eq. 1.

\[
\text{EWC} = \frac{(w_{sw} - w_d) \cdot 100}{w_{sw}} \tag{1}
\]

Here, \(w_{sw}\) and \(w_d\) are the weights of the sample in the swollen and dry state, respectively.

In some cases, the equilibrium swelling ratio (SR) and swelling degree (\(q\)) were calculated by Eqs. (2) and (3), respectively.

\[
\text{SR} = \frac{(w_{sw} - w_d) \cdot 100}{w_d} \tag{2}
\]

\[
q = \frac{w_{sw}}{w_d} \tag{3}
\]

Using the aforementioned relationships in Eq. (1), the EWC was recalculated from SR and \(q\), e.g. in Ref. \[35, 43\] as shown in Eq. (4) and (5).

\[
\text{EWC} = \frac{\text{SR}}{\text{SR} + 1} \tag{4}
\]

\[
\text{EWC} = \left(1 - \frac{1}{q}\right) \cdot 100. \tag{5}
\]
NANO- AND MICRO-PARTICLE REINFORCED COMPOSITE HYDROGELS

Different from hydrogels mentioned in the previous section, another approach to achieve structures with mechanically improved properties is to reinforce SN with appropriate fillers. Liu et al. [47] introduced reinforcement by Laponite XLG (Clay-G, Gn) (disk-shaped plates have d = 30 mm and t = 1 mm, respectively, and exhibit hydrophilic character) and Laponite XLS (Clay-S, Sm). The route started by the preparation of an aqueous solution of N-isopropylacrylamide (NIPAAm) as monomer and Clay-G or Clay-S as crosslinker. Potassium persulfate (KPS) as initiator and N,N,N′,N′-tetramethyldiamine (TEMED) as accelerator were then injected then into the mixture at 0 °C. After three days of polymeri-
zation time at 5 °C, a series of nano-particle reinforced composite hydrogels expressed by Sm and Gn (see Table 2) were obtained.

Nanocomposite (NC) hydrogels, using Laponite XLG were reported also in a series of works [48–51]. Aqueous solutions consisting of 1 mol/L NIPAAm as monomer and 10⁻³–2.5×10⁻¹ mol/L clay concentration were prepared. To achieve NC hydrogels with a concentration of clay 10⁻¹ mol/L and higher, a series of steps were performed. In the first step, an aqueous solution of NIPAAm containing a certain amount of clay was stirred. The residue of the clay was added afterwards while stirring at 1 °C and subsequently heating at 35 °C followed by cooling down to 1 °C in order to avoid flocculation and accelerated dispersion. Exfoliation of the clay and good dispersion of the components were obtained by further mixing for 30 minutes at 1–5 °C, applying revolution speeds of 2000/800 and 2200/60 rpm, respectively. After that the KPS as initiator and TEMED as accelerator were added and free radical polymerization took place for twenty hours at 20 °C.

A hydrophilic biodegradable polymer (HYAFF11) as a matrix and α-tricalcium phosphate (α-TCf) as a reinforcement were used to prepare HYAFF11/α-TCf composite hydrogels [52]. A HYAFF11 was obtained by a full esterification of the carboxylic groups of the Hyaluronic Acid (HyA) with benzyl alcohol, while α-TCf was obtained by heating in air for 15 hours at 1300 °C and subsequently quenching to ambient temperature in air of mixture of calcium hydrogen phosphate (CaHPO₄) and calcium carbonate (CaCO₃). HYAFF11/α-TCf composite hydrogels with 93 wt.% α-TCf and 86 wt.% α-TCf, respectively, were produced by mixing of 8 wt.% solution of HYAFF11 in dimethylsulfoxide (DMSO), and the calculated amounts of fillers were added until achieving a homogeneous composition. After 24 hours, some of the samples were dried at ambient temperature, while the others were immersed in distilled water for 24, 48 or 96 hours at 37 °C.

A PNIPAAm hydrogel reinforcing polyurethane foam (Polyurethane/PNIPAAm), given a hydrogel composite, was reported by the group of Liu and collaborators [53]. A 1,4-Dioxane solution containing 1 mol/L NIPAAm as monomer, 0.1 mol/L ethylene glycol dimethacrylate (EGDMA) as crosslinking agent and 0.036 mol/L 2,2′-azobisisobutyronitrile (AIBN) as initiator was first bubbled with nitrogen for 10 minutes and subsequently polymerized within the polyurethane foam for four hours at 55–60 °C.

Huang et al. [54] described macromolecular microspheres composite (MMC) hydrogels. Macromolecular microspheres (MMS) emulsions exhibit a mixture of monomers of styrene, butyl acrylate and acrylic acid (AA) as well as emulsifier and initiator. First, MMS emulsion was irradiated with ⁶⁰Co γ-rays in the presence of oxygen for two hours at ambient temperature whereas peroxides were generated on the surface and inside of the MMS. In the second step, different amounts of AA monomer and water were added and the mixture obtained was polymerized for six hours at 40 °C.

Hydrophilic reactive microgels (HRM) were presented by Qin et al. [55]. Different steps for the preparation of hydrophilic reactive microgel (HRM) hydrogels were performed. In the first step, hydrophilic microgels (HM) were synthesized, subsequently followed by the formation of hydrophilic reactive microgels (HRM). The HRM obtained were dispersed in a mixture of AA with few drops of octylphenol ethoxylate. After stirring for six
hours and bubbling with nitrogen for 40 minutes, definite amounts of AMPS as monomer, ammonium persulfate as initiator and TEMED as accelerator were added. Finally HRM hydrogels were achieved by polymerization for 48 hours at 20 °C.

Innovative poly(N,N-dimethylacrylamide) (PDMA) - silica hybrid hydrogels were introduced to assess the character of the effective physical interactions among silica nanoparticles and PDMA on the performance of chemically cross-linked PDMA networks [56], swollen in equilibrium state. In each case, PDMA-silica hydrogels were synthesized by combination of DMA and MBAA in molar ratios (n) 100:1, as well as DMA, KPS and TEMED = 100:1:1, respectively. The amount of silica nanoparticles dispersed in aqueous solutions (Ludox SM30) differed in the specimen. The classification of hydrogel samples was established on the volume fraction of silica to DMA multiplied by factor of 100 (Si0–Si100).

An original approach was developed to invent thermo-responsive poly(N-isopropylacrylamide) (PNIPAM) hydrogels (TPHs), cross-linked by biodegradable starch-based nanoparticles (SN) [57]. Initially, 0.5 g of acylated allylic starch (AAS) (Mw = 180000 g/mol) was dispersed in 20 ml of acetone. Subsequently, 10 ml of distilled water was added dropwise, and the obtained nanosphere suspension was stirred at ambient temperature up to a complete evaporation of the acetone. In the second step, 1 g of NIPAM and 0.005 g of 1-hydroxy cyclohexylphenyl ketone (IR 184) as photo initiator was supplemented to 9.5 g aqueous solution containing 0.5 g SN. The combination was cast in the tube, deaerated by nitrogen and photopolymerized for 12 hours at 365 nm at 0 °C.

Recently, biocompatible poly(ethylene glycol) diacrylate (PEGDA)/Laponite XLS NC hydrogels were described by Chang and collaborators as an attractive candidate to enhance the mechanical properties of hydrogel materials [58]. Laponite XLS was distributed in distilled water and retained at ambient temperature for 20 minutes in order to obtain a transparent solution. Consequently, Laponite XLS, 10 wt. % solution of PEGDA and Igracure 2959 as photo initiator (0.05 wt. % absolute concentrations) were brought together in nearly 20 minutes. The composition was then polymerized for 5 minutes at 365 nm. A sequence of PEGDA NC hydrogels consisting diverse amounts of Laponite XLS nanoparticles were synthesized by photopolymerization of 10 wt. % PEGDA (Mw = 10 kDa) and 0, 2.5, 5 and 10 wt. % Laponite.

By applying the freeze-thaw cycles, Wang et al. created hydrogel composites by the integration of distinctive quantities of PVA in transparent BC polymer template [59]. A BC layer (t = 0.3 mm) was immersed in aqueous solution of PVA in water bath at 80 °C during 24 hours, and subsequently frozen at –20 °C for 24 hours and thawed at ambient temperature for 12 hours. The performances of BC/PVA composite hydrogels which possess distinct BC content - 0, 12, 17, 27 wt. % were determined.

Tetra-poly(ethylene glycol) (PEG)-based nanocomposite hydrogels (tP-NC) were synthesised by the polymerization of macromonomers - tetraamine- and tetra-NHS-glutarate-terminated PEG (TA-PEG and TN-PEG) in the presence of clay (Laponite XLG) in aqueous solution [60]. Prior to reaction of TA-PEG and TN-PEG, the inorganic clay was combined by the macromonomer through distinctive methods: (a) clay and TA-PEG
mixed together, subsequently by TN-PEG, (b) contrary – clay combined by TN-PEG and consequently by TA-PEG, (c) partial amounts of clay by TA-PEG and TN-PEG, respectively, and the composition obtained was integrated.

Further, in case of tP-NC hydrogels achieved in presence of pyrophosphate-Na buffer, the synthetic route was different. Originally, transparent suspension of 0.3 g inorganic clay and 15 ml 100 mM buffer (pH 7.4) was prepared. Subsequently, 1.2 g TA-PEG was appended to the clay suspension, and the composition was stirred at ambient temperature continuously for 15 minutes (solution I). 1.2 g of TN-PEG was separately dispersed in 5 ml of 100 mM buffer (pH 7.2) (solution II). The obtained solution I, respectively solution II were combined at 4 °C, and the polymerization proceeded at ambient temperature (~25 °C) for 2 h. Depending on the mixing process, the hydrogels were denoted as tP-NCnpy-A, tP-NCnpy-N and tP-NCnpy-AN, where subscripts of n designate the clay concentration (c_{clay}) and the buffer in the reaction solution (py designates pyrophosphate-Na and ph – phosphate-Na), respectively. The c_{clay} was changed among 1.10^{-2} ≤ n ≤ 20.10^{-2} mol/L although the weight ratio of TA-PEG/TN-PEG was held constant - 1:1. The polymer concentration (c_p) was kept usually at 120 mg/mL, however in some cases altered among 80–240 mg/mL in order to study the effect of cp on the tensile properties.

Mechanically strong NC hydrogels composed of PEG and hydroxyapatite nanoparticles (nHAp) were recently reported in the literature as a desirable candidate in orthopaedic tissue engineering applications [61]. At first, the required amount of nHAp was dispersed in 0.4 % solution of initiator – Igracure 2959, subsequently dissolved by vortex for 10 minutes and sonicated for at least 20 minutes. The hydroxyl groups of PEG (M_w = 35 000 g/mol) were previously acrylated and the compound obtained was added to the nHAp solution. After combining for 20 minutes and consequent centrifugation, the composite solution was photopolymerized for 10 minutes at 365 nm, though the distance among specimen and UV lamp was held – 15 mm.

Wang et al. prepared a layered poly(N-isopropylacrylamide)-nanoclay (PNIPAM-nanoclay) hydrogel that shows a nacre-like structure [62]. The mixture of NIPAM, nanoclays and initiator were arranged in a structure of layers by the aid of vacuum filtration and subsequently polymerized through UV light to achieve layered-nanocomposite (L-NC) gels. As an inorganic clay was cho sen Laponite XLG ([Mg_{5.34}Li_{0.66}Si_{8}O_{20}(OH)_{4}]Na_{0.66}), and the initiator was 2,2’-Diethoxyacetophenone (DEOP).

Nanoclay-reinforced hydrogels based on a matrix of either poly(N-isopropylacrylamide) (PNIPA) or PNIPA/poly(N-vinylpyrrolidone) (PVP) as semi-interpenetrated network (SIPN) were introduced by Djonlagic et al. [63]. The aqueous polymer mixtures of each NIPA and NIPA/VP were just combined with nanoclays Laponite XLG as well as initiator ammonium persulfate (APS) and accelerator TEMED. The radical polymerizations occurred in each case at ambient temperature during 24 hours.

In Table 2, an overview of nanoparticle reinforced hydrogels is given, and additionally, values for the EWC are presented. The EWC for S_m and G_n hydrogels [47] as well as NC1–NC25 hydrogels [48] altered according to clay concentration, while in Ref. [53], EWC changed depending on the temperature of the PNIPAAm reinforcement. The EWC
Table 2. Overview of nano-particle reinforced composite hydrogels

<table>
<thead>
<tr>
<th>Research group</th>
<th>Ref. No.</th>
<th>Kind of hydrogel</th>
<th>EWC [%]</th>
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<tr>
<td>Liu et al.</td>
<td>[47]</td>
<td>Clay-S (S&lt;sub&gt;m&lt;/sub&gt;)&lt;sup&gt;1)&lt;/sup&gt; and Clay-G (G&lt;sub&gt;n&lt;/sub&gt;)&lt;sup&gt;11&lt;/sup&gt;</td>
<td>96.2–98.5 and 97.9&lt;sup&gt;2)&lt;/sup&gt;</td>
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<tr>
<td>Haraguchi et al.</td>
<td>[48]</td>
<td>NC1–NC25&lt;sup&gt;3)&lt;/sup&gt;</td>
<td>79–86.9</td>
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<td>Sanginario et al.</td>
<td>[52]</td>
<td>HYAFF11/α-TCP-x&lt;sup&gt;4)&lt;/sup&gt;</td>
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<tr>
<td>Liu et al.</td>
<td>[53]</td>
<td>Polyurethane/PNIPA</td>
<td>14–83</td>
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<tr>
<td>Huang et al.</td>
<td>[54]</td>
<td>MMC (A1–A6)&lt;sup&gt;5)&lt;/sup&gt; and MMC (B1–B4)&lt;sup&gt;6)&lt;/sup&gt;</td>
<td>70–89&lt;sup&gt;7)&lt;/sup&gt;</td>
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<tr>
<td>Qin et al.</td>
<td>[55]</td>
<td>HRM -x-y&lt;sup&gt;8)&lt;/sup&gt; and HM&lt;sup&gt;9)&lt;/sup&gt;</td>
<td>93.8–97.6 and 91.1</td>
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<td>Lin et al.</td>
<td>[56]</td>
<td>si(y)&lt;sup&gt;10)&lt;/sup&gt;</td>
<td>94.3–95&lt;sup&gt;11)&lt;/sup&gt; and 86.7–93.3&lt;sup&gt;12)&lt;/sup&gt;</td>
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<tr>
<td>Tan et al.</td>
<td>[57]</td>
<td>TPH</td>
<td>94.2–97.5</td>
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<td>Chang et al.</td>
<td>[58]</td>
<td>PEGDA&lt;sub&gt;x&lt;/sub&gt;NC&lt;sub&gt;y&lt;/sub&gt;&lt;sup&gt;13)&lt;/sup&gt;</td>
<td>75–96.2</td>
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<tr>
<td>Wang et al.</td>
<td>[59]</td>
<td>BC/PVA</td>
<td>67.4–83.4</td>
</tr>
<tr>
<td>Fukasawa et al.</td>
<td>[60]</td>
<td>tP-NC</td>
<td>—</td>
</tr>
<tr>
<td>Akhilesh et al.</td>
<td>[61]</td>
<td>PEG-nHAp</td>
<td>88.4–84.6&lt;sup&gt;14)&lt;/sup&gt;, 85.5–81.8&lt;sup&gt;15)&lt;/sup&gt; and 81.0–77.7&lt;sup&gt;16)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>[62]</td>
<td>PNIPAM-nanoclay</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PNIPA-nanoclay and</td>
<td>97.5</td>
</tr>
<tr>
<td>Djonlge et al.</td>
<td>[63]</td>
<td>PNIPA/PVP-nanoclay SIPN</td>
<td>98.0</td>
</tr>
</tbody>
</table>

<sup>1)</sup> Indexes m and n denote the ratio (clay/water) · 100
<sup>2)</sup> Weight of the dried gel, as prepared
<sup>3)</sup> Clay concentration, expressed by number
<sup>4)</sup> Concentration of α-TCF
<sup>5)</sup> The amounts of the AA and water was varied
<sup>6)</sup> Duration of the reaction time was changed
<sup>7)</sup> Water content [wt.-%] of gel at defined condition
<sup>8)</sup> x = wt-% of HRM with respect to the total weight of the as-prepared hydrogel; y = wt.% of the AMPS with respect to the AAm
<sup>9)</sup> 0.1 wt-% of MBAA as crosslinking agent and HM instead of HRM
<sup>10)</sup> y = 0, 25, 50, 75 and 100, respectively
<sup>11)</sup> q in pure water
<sup>12)</sup> q in 1 M NaCl
<sup>13)</sup> x designate the Mw of PEGDA, y - Laponite XLS concentration
<sup>14)</sup> EWC at 4 °C
<sup>15)</sup> EWC at 25 °C
<sup>16)</sup> EWC at 37 °C

varied for MMC (A1–A6) and MMC (B1–B4) hydrogels [54] as well as HRM-x-y [55] and BC/PVA hydrogels [59] depending on AA and reaction time [51], the content of HRM and AMPS [55] as well as the amount of BC [59]. In works [47] and [56–58] the EWC were recalculated using Eq. 5 although Eq. 4 was applied in Ref. [53].
MISCELLANEOUS HYDROGELS

In contrast to the previously mentioned two approaches, a strengthening of hydrogels can occur also through methods of blending the single network with other polymers, cross-linking copolymerisation and network chemical modification by Click chemistry reactions. Copolymer hydrogels of 2-hydroxyethyl methacrylate-stat-2-methacryloyloxyethyl N-butylcarbamate (HEMA-stat-MBC) were introduced from Mequanint et al. [64]. An aqueous solution of pure water (Milli-Q) consisting of HEMA and MBC as monomers and AIBN as initiator was first stirred in order AIBN to be solved, bubbled with nitrogen and subsequently sonicated for five minutes. After 24 hours of polymerization at 65 °C, four different kinds of hydrogels were synthesized in which the molar ratio of HEMA/MBC mole ratio was changed from 100:0 to 85:15.

Original PEG-based hydrogel materials synthesized by Click chemistry resulted in a structure which is characterized by extreme mechanical properties [65]. One structural component was obtained by esterification of hydroxyl-terminated PEG (M_n = 3.4, 6.0, 8.0 and 10 kDa) and 4-pentynoic anhydride in the presence of 4-dimethylaminopyridine, whereby the required acetylene-functionalized polymers (1) were produced. The supplementary constituent was prepared by esterification of tetraethylene glycol and anhydride of isopropylidene-2,2-bis(methoxy)-propionic acid, consequently followed by deprotection reaction via acidic resin in gentle conditions. The synthesized adduct was activated by mesyl chloride, subsequently by nucleophile substitution with sodium azide and as a result the tetraazide (2) was generated. Dual equivalents of the component (1) reacted with the constituent (2) at ambient temperature, whereas aqueous conditions in the presence of copper sulphate and sodium ascorbate as a reducing agent were applied. In consequence, a hydrogel material (3) was synthesized that retained diverse gel fractions. Malkoch et al. [65] reported that the efficiency of cross-linking is extremely affected by the concentrations of polymer and catalyst, respectively. Moreover, the properties of hydrogels (3) were compared to the traditional PEG hydrogels (4) obtained by photopolymerization of PEGDA (M_n = 3.4 and 14 kDa).

Wagner et al. synthesized hydrogels on the basis of an attractive class of copolymers, based on poly(NIPAAm[A]-co-AAc[B]-co-N-acryl-oxysuccinimide (NAS)[C]-copolylactide-hydroxyethyl methacrylate (HEMAPLA)[D] at 37 °C [66]. 10 wt. % Monomer solution of NIPAAm, AA, NAS HEMA-PLA in 1,4-dioxane was prepared and subsequently bubbled with argon gas for ten minutes. BPo as initiator with concentration 7.2×10^{-3} mol/mol monomer was added to the mixture. After polymerization for 24 hours at 70 °C, the polymer solution was cooled in order to reach an ambient temperature. After this, the product was precipitated in hexane, filtrated and dried under vacuum for one night. Furthermore, the copolymer was refined by precipitation twice in diethyl ether and the hydrogel obtained was dried for two days at 50 °C.

A series of physically blended chitosan/PEG/PNIPAAm hydrogels were reported from the group of Sun and collaborators [67]. A solution of NIPAAm as monomer in methanol and 2 mol % BPO as initiator were polymerized for 24 hours at 70 °C. The product obtained
was purified by precipitation in excess of diethyl ether, followed by dissolution in acetone and repeated precipitation in diethyl ether for three more times. After drying in vacuum at ambient temperature for 24 hours, PNIPAAm with molecular weight of 33,000 g/mol was obtained. Definite amounts of chitosan/PEG/PNIPAAm blends with different composition ratios were dissolved in solution of acetic acid with concentration of 2 v/v %. Furthermore, the mixture was stirred for two days, filtered in order to remove undissolved materials and consequently dried for two days, first in fume hood and after that under vacuum. The molecular weights of chitosan and PEG are 400,000 and 2,000 g/mol, respectively.

Cellulose hydrogels (CW) and cellulose/poly(ethylene glycol) (CP) gel membrane were described in a work [68]. A 5 wt. % solution of cellulose was dispersed in an aqueous solution of NaOH-thiourea, which was preliminarily cooled to 5 °C, and stirred for five minutes in order to achieve a transparent cellulose solution. The mixture obtained in this way was injected into square glass mask, stored for eight hours at –20 °C and subsequently thawed to ambient temperature. The cellulose physical gel prepared was put to swell in water in order for the remainders of NaOH and thiourea to be eliminated. CW hydrogels were immersed in PEG with various molecular weights for one week at 50 °C, and CP hydrogel membranes were produced.

For the preparation of PEU hydrogels a series of reactions were performed [43]. In the first step, azeotropic distillation in toluene of mixture of PEG with glycerol was made, followed by evaporation of the solvent residues on a rotary evaporator at 60 °C whereby PEG is melted. After that, a definite amount of IPDI was added in such a manner that the molar ratio of OH and NCO groups was to be 1:1. By applying a vacuum for one minute to remove the air and subsequent polymerization for 20 hours at 70 °C, PEU hydrogels were obtained.

An innovative type of hybrid hydrogel, obtained by the incorporation of a synthetic hydrogel inside the biological one directly isolated from an animal body is recently reported in the literature as an attractive candidate possessing significantly enhanced mechanical properties [69]. The jellyfish stated by Rhopilema esculenta Kishinouye and the slabs of jellyfish umbrellas were initially treated by combination of salt and alum. Subsequently, they were washed through deionized water for 72 hours in order to subtract the salt. The exterior skin of the umbrellas was gently eliminated and leaves the mesogloea as a raw material (Jf gel). For the preparation of hybrid hydrogels, the Jf gel was immersed in monomer solution of PAA, respectively PAAm in the presence/absence of cross-linking agent MBAA for 18 hours in order to ensure the complete exchange of water by the monomer solution and subsequently the irradiation by 60Co-γ rays for 2 hours.

Hydrogel materials demonstrating significant compression moduli, correspondingly stress-at-break are obtained by the approach of sequential IPN of densely cross-linked PEGDA and negligible cross-linked PAA [70]. In the initial stage PEGDA was dissolved in deionized water at concentration of 50 wt. %. The photo initiator – 2-hydroxy-2-methyl-propiophenone was supplemented to the solution in concentration 1 wt. % and the composition was photopolymerized at 365 nm for 5 minutes. In the second step, the synthesized PEGDA SN was immersed for 24 hours in 50 v/v-% solution of AA (pH = 1.7)
containing 1 v/v- % 2-hydroxy-2-methyl-propiophenone and 1 v/v- % triethylene glycol dimethacrylate as a cross-linking agent for 24 hours. Photopolymerization for duration of 5 minutes results in the development of a second PAA network cross-linked in the presence of previously created PEGDA SN.

The overview of hydrogels prepared and also their EWC are depicted in Table 3.

The EWC of HEMA\[^{[X]}\]-stat-MBC\[^{[Y]}\] and P(NIPAAm\[^{[A]}\]-co-AAc\[^{[B]}\]-co-NAS\[^{[C]}\]-co-HEMAPLA\[^{[D]}\] hydrogels \[^{[66]}\] chanced in correlation with the HEMA/MBC \[^{[64]}\] and NIPAAm/HEMAPLA molar ratio as well as AA content and number of lactate units \[^{[66]}\], respectively. In Ref. \[^{[43]}\] and \[^{[69]}\] the EWC depends on the amount of crosslinking agent and the molecular weight of PEG, correspondingly monomer concentration, while in Ref. \[^{[65, 67]}\], the molecular weight of PEG gives an impact to the swelling behaviour.

### MECHANICAL PROPERTIES

#### Mechanical properties of double network hydrogels

Types of testing methods, used parameters and obtained mechanical properties of hydrogels made out of DN are summarized in Table 4. All measurements were performed on hydrogels at their EWC.
Osada et al. presented tensile and compression test measurements of PAMPS-1-4/PAAm-2-0.1 DN hydrogels [24, 29–34, 37]. It was found that DN hydrogels sustained a tensile stress of 0.68 MPa and strain-at-break of 75%, while PAMPS-1-4 SN hydrogel broke down at 0.05 MPa and 6%. Under compression load, PAMPS-1-4 and PAAm-2-0.1 SN hydrogels broke at stresses of 0.4 MPa and 0.8 MPa, respectively, while PAMPS-1-4/PAAm-2-0.1 DN hydrogel sustained a stress of 17.2 MPa. The compression strain-at-break value obtained altered between 41 to 84% for PAMPS-1-4 and PAAm-2-0.1 SN hydrogels, and 92% for their DN hydrogel.

Osada and collaborators also reported that two structural parameters are responsible for achieving mechanically strong hydrogels: a) molar ratio of the second to the first network and b) crosslinking densities of both networks [24]. It was shown that the mechanical strength of DN hydrogels increased dramatically when the molar ratio of the second to the first network was in the range of several to a few tens.

In the works [24, 29–34, 71, 72], the effect of variation of the crosslinking density of PAAm second network on the mechanical properties of the final DN was reported. The crosslinking density of PAMPS SN was kept at 4 mol %, while the concentration of MBAA in the PAAm SN was altered between 0–3 mol %. In all cases, similar Young’s moduli and EWC values were obtained as 0.3 MPa and 90 %, respectively, while the molar ratio of the second network to the first network was constantly 1 to 20 (with respect to the change in the crosslinking density of the second network). Nevertheless, a dramatic change in the mechanical strength of the DN hydrogels was observed. The highest stress-at-break (10 MPa) was reported when the crosslinking density of the second network was kept at 0 mol %. Over a crosslinking density of 0.5 mol %, the fracture strength reached a constant minimum of 0.69 MPa.

In order to explain the mechanical strength of PAMPS-1-4/PAAm-2-0.1 DN hydrogels, Osada et al. proposed a structural model [71–73]. PAMPS-1-4 SN hydrogel is stiff, but brittle and inhomogeneous, consisting of large “voids” formed during the radical polymerization. When PAAm second network was polymerized in the presence of the PAMPS network, a part from PAAm chains were interpenetrated in the first network, while the other occupied the “voids”, partially entangled with the first network. Under compression load, the linear or poorly crosslinked PAAm in the “voids” effectively absorb the crack energy either by viscous dissipation or by deformation of the PAAm chains. The crack is excluded to grow to a macroscopic level, while the part of PAAm which is entangled with PAMPS SN can act as an “anchor”. As a consequence, the long PAAm chains can be elongated and subsequently damaged, consuming the crack energy.

Later on, Osada et al. reported that the mechanical properties of DN hydrogels were also affected from the molecular weight (M_w) of PAAm [73]. It was demonstrated by elemental analysis that for PAMPS-1-4/PAAm-2-0.1 DN hydrogel (remained to swell in water for at least one month), the PAAm chains with lower M_w diffused out of the gel and as a result, the molar ratio of the second to the first network decreased. Additionally, the result was given that the compressive fracture stress was enhanced significantly when the M_w is around 10^6 Da, while below this value, the stress-at-break did not change. In
Table 4. Mechanical testing of DN hydrogels – overview of methods and results

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Testing Method</th>
<th>Set of parameters</th>
<th>Results</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Stress-at-break</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$\sigma$ [MPa]</td>
</tr>
<tr>
<td>[24]</td>
<td>a) Tensile and</td>
<td>a) $L^{(1)} = 50$, $b^{(2)} = 5$, $s^{(3)} = 3$, $v^{(4)} = 10$</td>
<td>a) 0.68</td>
</tr>
<tr>
<td></td>
<td>b) Compression</td>
<td>b) $d^{(5)} = 9$, $s = 4$, $v = 0.1$</td>
<td>b) 17.2</td>
</tr>
<tr>
<td>[25]</td>
<td>Compression</td>
<td>$d = 9$, $s = 4$, $v = 10$</td>
<td>3.1</td>
</tr>
<tr>
<td>[25]</td>
<td>Compression</td>
<td>$d = 10$, $s = 5$, $v = 10$</td>
<td>2.9</td>
</tr>
<tr>
<td>[25]</td>
<td>Compression</td>
<td>ditto</td>
<td>3.7</td>
</tr>
<tr>
<td>[35]</td>
<td>a) Tensile and</td>
<td>a) $L = 12$ and $7$, $b = 2$ and $10$, $v = 10$</td>
<td>a) 3.5</td>
</tr>
<tr>
<td></td>
<td>b) Compression</td>
<td>b) $d = 10$, $v = 10$</td>
<td>b) 6.5</td>
</tr>
<tr>
<td>[36]</td>
<td>a) Tensile and</td>
<td>a) $L = 40$, $b = 10$, $s = 1.7$–$2.6$, $v = 100$</td>
<td>a) 0.8</td>
</tr>
<tr>
<td></td>
<td>b) Compression</td>
<td>b) $d = 9$, $s = 5$, $v = 10$</td>
<td>b) -</td>
</tr>
<tr>
<td>[26]</td>
<td>Compression</td>
<td>$d = 9$, $s = 3$, $v = 10$</td>
<td>9.2</td>
</tr>
<tr>
<td>[26]</td>
<td>Compression</td>
<td>$d = 9$, $s = 4$, $v = 10$</td>
<td>4.8</td>
</tr>
<tr>
<td>[38]</td>
<td>Compression</td>
<td>$a = b = 5$, $s = 1$ or $d = 9$, $s = 2$, $v_1 = 1$</td>
<td>1.6–2.3</td>
</tr>
<tr>
<td>[39]</td>
<td>Tensile</td>
<td>$L_1^{(7)} = 9.5$, $b = 3.18$, $s = 0.25$–$0.75$, $v_2 = 15$</td>
<td>0.86–12.8</td>
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<td></td>
<td></td>
<td></td>
<td>$\text{Standard ASTM D638}$</td>
</tr>
<tr>
<td>[40]</td>
<td>Compression</td>
<td>$d = 5$, $s = 5$, $v_2^{(8)} = 1$</td>
<td>0.442±0.11–0.54±0.06</td>
</tr>
<tr>
<td>[41]</td>
<td>Compression</td>
<td>$d = 8$, $s = 5$, $v_1 = 0.1$</td>
<td>5.25</td>
</tr>
<tr>
<td>[42]</td>
<td>a) Tensile and</td>
<td>a) $L = 65$, $b = 5$, $s = 2$, $v = 5$</td>
<td>a) 4.12–6.10</td>
</tr>
<tr>
<td></td>
<td>b) Compression</td>
<td>b) $d = 25$, $s = 25$, $v_1 = 5$</td>
<td>b) 12.76–5.15, 0.087–0.718 $^{(10)}$</td>
</tr>
<tr>
<td>[43]</td>
<td>Compression</td>
<td>$d/s = 2/1$, $d = 25$, $d^{(9)} = 10$, $v_1 = 0.5$%/s</td>
<td>3.2±0.5–3.5±1.1</td>
</tr>
<tr>
<td>[44]</td>
<td>a) Tensile and</td>
<td>a) acc. JIS-K6251–7, $v_1 = 100$</td>
<td>a) 1.95</td>
</tr>
<tr>
<td></td>
<td>b) Compression</td>
<td>b) $d = 9$, $s = 4$, $v = 10$</td>
<td>b) —</td>
</tr>
<tr>
<td>[45]</td>
<td>Tensile</td>
<td>$v_2 = 200$</td>
<td>0.6</td>
</tr>
<tr>
<td>[46]</td>
<td>Compression</td>
<td>Cylindrical, $v_1 = 0.3$</td>
<td>7</td>
</tr>
</tbody>
</table>

1) Length L of specimen in [mm]
2) Width b of specimen in [mm]
3) Thickness s of specimen in [mm]
4) Strain or compression rate v [%/min]
5) Diameter d of specimen in [mm]
6) Compression or tensile speed $v_1$ [mm/min]
7) Gauge length $L_1$ of specimen in [mm]
8) Cross-head speed $v_2$ [mm/min]
9) Displacement $\delta$ [%]
10) Effects of freezing and thawing cycles
all cases, compression moduli of DN hydrogels remained to a constant value of 0.3 MPa, regardless of the M_w of PAA.

Recently it was reported the influence of the modification of the PAMPS SN (either by altering the MBAA concentration or by introducing γ-ray radiation as a crosslinking method) on the DN hydrogels’ mechanical properties [74–76] was reported. Under elongation, the tensile stress increased uniformly with the increase of the strain. When a critical value of 0.21 MPa stress was reached, a “neck” appeared in the gel and grew up with further deformation. It was reported that the necking deformation happened between 2–8 % extensions, and as a result, the DN hydrogels became soft. The Young’s modulus of hydrogel after necking (Es) was approximately 0.015 MPa, while before necking (Eh), nearly 0.1 MPa was reached (Es and Eh were determined by compression tests). The demonstration of the yielding deformation was exhibited at the plateau of the stress-strain curve of DN hydrogels.

Several loading curves of repeated elongation tests for PAMPS-1-2/PAAm-2-0.02 DN hydrogels were performed. During the first cycle, the hydrogels were elongated to a length at which the “neck” appeared and subsequently remained to relax to its original form. In the second load step, the sample was stretched up to the same length as during the first cycle. But a deformation of the softened neck region occurred, and as a result, the stress-strain curve followed different ways like at the beginning. From third to fifth loadings, the stress-strain curves overlapped with each other, so that, the deformation can be assumed to be rubber-elastic. During the sixth loading, a stretching curve beyond aforementioned curves was observed, while in the time of the seventh loading the DN hydrogels were elongated until the breaking point appeared. Osada et al. proposed that during the yielding deformation, the PAMPS SN broke into small pieces which can act as crosslinker for the PAAm chains. As proposed in works [71–73], the inhomogeneity of the PAMPS SN introduced by radical polymerisation gave influences on the mechanical strength of the DN hydrogels. However, the mechanism for this behaviour was not explained in a detailed manner. The existence of necking could give a concept to explain the phenomenon: If the necking deformation occurs in the mesoscale region around the crack tip of the DN gels, the stress concentration is reduced resulting in a larger toughness.

Gong et al. [77] reported that the yielding deformation appeared also in PAMPS-1-4/PAAm-2-0 DN hydrogels. The different amount of initiator during the second polymerization (0.005 mol %) as well as the lack of more precise conditions explained the absent of “neck” zone in the PAMPS-1-4/PAAm-2-0.1 DN hydrogels. Using atomic force microscopy (AFM) the Young’s modulus below the fracture surface (E_f) and below the usual moulded surface (E_m) was determined. The values achieved with these for the Young’s modulus measured before and after the yielding deformation, depicted as Eh and E_h were compared. It was observed that E_m and E_h were in magnitude of 0.1 MPa, while the E_f and E_a ranged from 0.001 to 0.01 MPa, which proved the existence of the yielding around the crack.

Gong and collaborators also divided the tensile behaviour of the elements into three groups. An element in the chemical gel was assumed to be the sub-chain between two crosslinking points, while in the DN hydrogels the element included several PAMPS
clusters obtained by yielding deformation. Brittle materials, which behaved elastically at small strains and subsequently broke down at stretching, belong to the first class. Elements undergoing the yielding deformations before the damage, related to the second group (for example, amorphous polymer consists of short chains), while the third group corresponds to elements undergoing yielding and subsequently re-hardening (DN hydrogels). Because of the re-hardening of the element around the crack, the elements around it sustain yielding, before it breaks down. It was found that the dissipative yielding zone around the crack was quite broad; and as a result, high fracture energy was generated.

Even if the aforementioned models could explain the extremely high mechanical strength of DN hydrogels, one question still remained, namely, why the strongest hydrogels were obtained when during the second polymerization no crosslinking agent was used [78]. In the work of Gong et al., the existence of two types of DN hydrogels were reported – connected (c-DN) and truly independent (t-DN) DN hydrogels. The c-DN sustained extremely high compression strength and strain-at-break – 7.66 MPa and 88 %, respectively, while the t-DN hydrogels broke down at stress of 0.37 MPa. The authors announced that the c-DN, PAMPS SN and PAAm SN were connected covalently by copolymerization of PAAm with the remainder of MBAA in PAMPS SN. During compressive behaviour, the stress loaded to the DN hydrogel was transferred from the first network to the second one by interconnected structure as mentioned above.

Compression test measurements were performed on PAMPS-1-4/PDMAAm-3.0-0.1, BC/PDMAAm-3.0-0.1 and BC/Gelatin-30wt. %-1.0 DN hydrogels, as reported in works [25, 27–28, 32, 34]. The maximum compression strength obtained for the aforementioned DN hydrogels was 3.1 MPa, 2.9 MPa, 3.7 MPa, respectively, while the values achieved for the strain-at-break altered as follows: 73 %, 50 % and 37 %. The initial compression moduli were found to be 0.2 MPa, 1.6 MPa and 1.7 MPa, respectively. Furthermore, the mechanical properties of DN-L and TN were presented in Ref. [26, 34]. DN-L sustained compression strength and strain-at-break of 9.2 MPa and 70 %, while the TN broke down at 4.8 MPa stress and 57 % strain, respectively.

The mechanical properties of BC/PAAm-x2-y2 DN hydrogels were evaluated [35]. In case of BC/PAAm-x2-0.1 DN hydrogels, the compression stress-at-break enhanced among 2.5–4.25 MPa at concentrations 1 to 4 M, although the EWC and strain-at-break declined. The influence of AAm concentration on the tensile strength of DN hydrogels is analogous to the effect exposed in compression tests, for instance, the highest fracture stress and corresponding slightest elongation - 2.75 MPa and ~1 mm/mm were obtained at concentration of 4 mol/L.

The change in the cross-linking density on the compression and tensile properties of BC/PAAm-4.0-y2 DN hydrogels was further investigated [36]. A considerable increase in the compression and tensile stress-at-break was achieved at MBAA concentrations up to 1 mol %, although at cross-linker concentration beyond 2.5 mol %, the changes are almost persistent. In contrast, the mechanical strain-at-break declined significantly above concentrations of 1 mol %. The obtained extreme compression and tensile fracture stress/
strain were 3.5 and 6.5 MPa, and correspondingly ~1.3 mm/mm and ~0.45 mm/mm at concentrations of MBAA – 5 mol % and 1 mol %. The effect of BC possesses a controlled water content on the mechanical performances of BC/PAAm-4.0-2.5 DN hydrogels.

Gong et al. considered the mechanical properties of PVA SN in order to explain the formability and the strength of PVA-DN hydrogel, respectively [36]. The PVA SN hydrogel demonstrated significant elasticity and absence of fracture, even though strain of 10 mm/mm occurred. Supplementary, the determined stress-at-break is considerable. In consequence, the PVA SN hydrogel preserved its original structure despite being separated from the template. On the contrary, the conventional PAMPS SN hydrogel exhibited distinctive performances, as rigid and fragile behaviour which sustains stress of 0.08 MPa. The authors indicated the elasticity as a crucial parameter responsible for the formability. The considerable mechanical properties of PAMPS-1-4/PAAm-2-0.1 DN hydrogels they related to the rigidness of the PAMPS-1-4 SN hydrogel. In order to evaluate the degree of stiffness, the quantity of compression modulus was assessed. The PAMPS-1-4 SN hydrogel demonstrated a compression modulus on a high value nearly at 0.25 MPa, although PVA-PAMPS hydrogel displayed ~0.15 MPa. In consequence, despite the presence of PVA SN, the performance of PVA-PAMPS hydrogel was determined by the rigid PAMPS-1-4 SN which caused the toughening of DN hydrogels. In contrast, PVA SN hydrogel possesses negligible compression modulus ~0.05 MPa, though the concentration is as equal in PAMPS-1-4 SN hydrogel. Therefore, the PVA/PAAm DN hydrogels exhibited no high toughness.

The stress-strain behaviour of PVA-DN, respectively PAMPS-1-4/PAAm-2-0.1 DN hydrogels were also compared [37]. The tensile strength of PVA-DN hydrogel was negligible (0.8 MPa) although the fracture strains were analogous. The described effect was assigned to the decreased concentration of PAMPS inside of the PVA-DN and PVA-PAMPS hydrogels. However, by applying of higher concentrations of PAMPS, the effect of PVA chains can be overcome. Gong et al. presented DN hydrogels which exhibit contemporaneous rigidity and flexibility, and enable applications in biological and industrial areas, e.g. implantation of artificial cartilage in patients, design of artificial blood-vessels with complicated shapes, and gel machines established on chemo-mechanical systems.

In the work of Ajiro et al. [38], the compression strengths of NVA-X-1 SN hydrogel (X = concentration of NVA = 2, 4 mol/L) and NVA-2-1/NVA-2-1 DN hydrogels were compared. NVA-2-1 and NVA-4-1 SN hydrogels sustained stresses of 0.51 MPa and 1.3 MPa as well as 0.33 MPa and 1.4 MPa, respectively, while the NVA-2-1/NVA-2-1 DN hydrogels broke down at 1.6 MPa and 2.3 MPa. These values changed depending on the thickness of the silicon gasket used at the time of the polymerization. An explanation about the mechanical strength of DN hydrogels was not presented from the authors.

Tensile measurements on PEG-DA(X) and PAA SN hydrogels as well as PEG-DA(X)/PAA[Y] DN hydrogels were performed by Myung et al. [39]. Three different parameters were varied (see below a, b and c), and their influences on the mechanical strength of hydrogels were reported in the following sub-chapters.
a. Molecular weight (Mw) of PEG-DA

The Mw of PEG-DA was altered from 3.4, 4.6, 8 and 14 kDa, while the acrylic acid polymerization conditions were kept invariable (and in fact, aqueous solution of 50 v/v % AA, containing 1 v/v % initiator and 1 v/v % crosslinking agent). The stress-strain profile of PEG-DA(X)/PAA DN hydrogels was presented. The tensile strength of PEG-DA(X)/PAA DN hydrogels with Mw of PEG-DA 3.4, 4.6, 8.0 and 14 kDa was found to be 9, 8.5, 2.5 and 0.25 MPa, while the strain-at-break changed to 0.6, 0.9, 1.0 and ~0.78 %, respectively.

To compare the mechanical properties of PEG (8.0 kDa) and PAA SN hydrogels as well as PEG-DA (8.0 kDa)/PAA DN hydrogels and their copolymers showing different EWC, the stress data was normalized on the base of polymer content in the gel. SN hydrogels PEG-DA (8.0kDa) and PAA sustained stresses of 1.5 and ~1.0 MPa, while PEG-DA (8.0 kDa)/PAA DN hydrogel and its copolymer broke down at 10.6 and ~1.2 MPa. The corresponding strains are as follow: 0.6, ~0.98, ~1.05 and ~0.75 %. The Young’s moduli (Eo) of PEG-DA SN, the copolymer and DN hydrogels were identical namely 0.91 MPa, while Eo of PAA SN reached a lower value to 0.55 Mpa [39].

b. Polymer content of PAA in the second network

In this case the Mw of PEG-DA was kept constant to 3.4 kDa, while the volume fraction of AA at solution was changed between 0.5 and 0.8 kDa before polymerization. It was reported that the EWC of PEG-DA(3.4kDa)/PAA[Y] DN hydrogels decreased with increasing the concentration of AA, while the mechanical strength and Eo grew up, e.g. the tensile stress-at-break reached for Y = 0.5, 0.7 and 0.8 values of ~4.5, ~12.5 and ~12.8 MPa, respectively, while the moduli were reduced in the same order of 3.6, 12 and 19.6 Mpa [39].

c. pH influence on the mechanical strength of DN hydrogels

The PAA SN and PEG-DA(8.0 kDa)/PAA DN hydrogel were put after the synthesis to swell in buffer solutions with pH 3–6 and constant ionic strength (I) of 0.05. It was notified that tensile strength of PAA SN and PEG-DA(8.0 kDa)/PAA DN hydrogel at pH 3 was 0.38 and 8.2 MPa, while pH 6 gave 0.05 and 0.86 MPa, respectively. The values for strain-at-break of hydrogels were approximately the same, as follows ~1.2 % at pH 3 and ~0.55 % at pH 6. The Eo of PAA SN hydrogel revealed a small drop from 0.09 to 0.05 MPa when the pH value increased from 3 to 6, while it did not change significantly for PEG-DA(8.0 kDa)/PAA when pH was varied from ~0.5 to ~0.7 MPa. When pH value increased from 4 to 5, Eo of PAA SN hydrogel dropped down, while the tendency for PEG(8.0 kDa)-DA/PAA DN hydrogels was contrary. The decreasing value of Eo for PAA SN correlated with an increase in the EWC of the PAA network and loss of hydrogen bonds [39].

To explain the mechanical strength of PEG-DA(X)/PAA[Y] DN hydrogel in comparison with PEG-DA(X) and PAA[Y] SN hydrogels, Myung et al. [39] clarified the effect of interpolymer hydrogen bonds and the impact of the interpolymer entanglement.

As aforementioned, the tensile strength, strain-at-break and Young’s modulus of DN hydrogels depends on the pH of the swelling liquids. At pH 3 and pH 4 (PAA, pKa = 4.7), the PAA SN is protonated and contracted, while at pH 5 and pH 6, the PAA network is negatively charged and swollen. DN hydrogels of PEG-DA(X)/PAA[Y] placed to reach
equilibrium in buffers with pH 3 and pH 4, respectively, sustained approximately twice higher stresses and extensibility than hydrogels at pH 5 and pH 6. The loss to elongate at high pH values was demonstrated by the lower strain-at-break of PAA SN compared with this reached at pH 3 and pH 4. Because of this, the participation of ionized PAA to “support” PEG was lost and the “polymer-diluting” effect of swelling decreased its strength [39].

The normalization of polymer content disclosed that the tensile stress-at-break and strain-at-break increased around two times when the pH decreased. It is known that hydrogen bonds can be created between ether oxygen’s in PEG and carboxylic acid groups in PAA, which explained the results from the normalization. Myung and collaborators [39] reported that they can play an important effect to support the entirety of the network through opposition to the crack dissipation during deformation and act like a source for strain hardening at high strains, since the PEG and PAA functional groups are more approachable when their chains are extended.

In order to explain the effect of strain hardening at low pH, the same authors relay it to the supposition of „entanglement reinforcement“ by interpolymer complexation between PEG-DA/PAA in solution and in IPNs, as presented from Iliopoulos and collaborators [27]. The theoretical model presented from Iliopoulos et al. suggested that PEG and PAA complex together along the segments of complementary sequences. As aforementioned that a PEG-DA(X)/PAA[Y] DN hydrogel is placed at pH 3 and pH 4, hydrogen bonds between PAA carboxylic acid groups and PEG ether oxygen were formed. These physical crosslinks can be reinforced by interpolymer hydrogen bonds between both monomers at entangled points. As a result, series of entanglements strengthened by hydrogen bonds were built. In comparison with inter-chain interactions, intra-chain interaction within the DN hydrogels has a small participation.

Unlike pH 3 and pH 4, at high pH hydrogen bonds play a little or no effect in strength enhancement. As aforementioned at pH 5 and pH 6, the PAA SN is negatively charged and swollen. However, the presence of PEG causes stronger entanglement (“pre-stressed state”) between both SN in the overall PEG-DA(X)/PAA[Y] DN hydrogel, because PEG SN elicits steric hindrance to PAA SN swelling. As a result, DN hydrogels had enhanced Young’s moduli due to not so much to the hydrogen bonds than the stronger entanglements. It was recorded that the effect of “pre-stressed state” depends on the relative amount of PAA in the second network. An increase in the PAA amount enhances the interactions with PEG SN, which results in a greater degree of pre-stresses in the DN and significantly higher modulus.

Compression measurements of chemical SBMA-2.5-81 SN hydrogel and chemical-physical polySBMA/SBMA-2.5-81 DN hydrogels were performed, as given in the Zhang et al. work [40]. The stress-at-break, strain-at-break and compression modulus of SBMA-2.5-81 SN hydrogel was 0.350±0.108 MPa, 0.42±0.02 % and 0.269±0.084 MPa, respectively. In comparison, the mechanical properties obtained for DN (1:28) and DN (1:139) (1:28 and 1:139 correspond to the ratio of polySBMA to SBMA monomer in wt. %) are as follows: fracture stress 0.442±0.117 and 0.595±0.094 MPa; fracture strain 0.54±0.06 and 0.57±0.02 %; initial moduli 0.179±0.032 and 0.198±0.016 MPa. The authors explained
the higher fracture stress of DN hydrogels in comparison with the SN hydrogels with the physical crosslinking points formed by the incorporation of linear polySBMA into the chemical SBMA SN, which can absorb more energy when deformation occurred. The fracture stresses of polySBMA/SBMA-2.5-81 DN hydrogels with different M_w of linear polySBMA were compared. An addition of polySBMA with M_w of 20.9 kDa and 169 kDa can increase the compression stress-at-break of DN twice as much in comparison with the SN hydrogel, while a further increase in the M_w to 203 kDa did not lead to an improvement of the fracture stress. This result shows that more defects at higher M_w can be introduced by incorporating the linear polySBMA.

The compression fracture stress and strain for PHA and D-3-0.05 SN hydrogels as well as PHA/PDMAAm-3-0.05 DN hydrogels were presented in the Weng et al. work [41]. The PHA and D-3-0.05 SN hydrogels fractured at stresses of 0.29 and 0.04 MPa, correspondingly, while PHA/PDMAAm-3-0.05 DN hydrogel sustained a stress of 5.25 MPa. The compression strain-at-break measured for PHA and D-3-0.05 SN hydrogels was 56.1 and 78.4 %, respectively while the fracture strain of PHA/PDMAAm-3-0.05 DN hydrogel was relatively more higher at 87.1 %. The initial compression moduli of PHA SN hydrogel and PHA/PDMAAm-3-0.05 DN hydrogel were deduced from the stress-strain curves, and they were found to be 0.045±0.005 and 0.508±0.08 MPa. Two parameters of the synthesis were varied, as follows: a. DMAAm concentration and b. MBAA concentration. Additionally, their influences on the mechanical properties were studied. In the first case (a), DMAAm concentration was changed from 0 to 3 mol/L, resulting in an increase of the compression stress-at-break from 0.29 to 4.12 MPa, however, a further increase in DMAAm concentration led to a considerable decrease in the fracture stress. In the second case (b), DMAAm concentration was kept at 3 mol/L, while the concentration of MBAA was altered from 0 to 2 mol %. The highest stress-at-break was achieved for PHA/PDMAAm-3-0.05 DN hydrogels, and in fact 5.25 MPa, which can not be explained with an increase in the chemical crosslinking or physical entanglement because the PDMAAm second network was poorly crosslinked.

In the same work [41], PHA SN is highly crosslinked and brittle, while the D-3-0.05 SN is loosely crosslinked and exhibits ductile behaviour. Cracks in the PHA SN could be generated by the stress applied under compression conditions. The presence of PDMAAm SN, however, could increase the fracture stress despite the stress by its deformation and/or sliding of the physical entanglement points along the polymer chains. When the concentration of MBAA increases from 0.05 to 2 mol %, the fracture stresses of the hydrogels decreases. Enhancing the crosslink concentration resulted in formation of a stiffer network with limited capacity of dissipation of the stress during compression.

The measurements of tensile and compressive stress and strain for water-swollen PVA-6-3 SN hydrogel and PVA-6-3/PEG-x2-3 DN hydrogels (x2 = 2, 4 and 6, respectively) were reported in the Zhang et al. work [42]. The tensile strength and Young’s modulus increases from 1.51 and 0.03 MPa for pure PVA-6-3 hydrogel to 6.10 and 0.16 MPa for PVA-6-3/PEG-2-3 DN hydrogel, which proved a considerable increase in the mechanical strength when an amount of PEG was added. The compression stress at break and compresen
sion modulus were compared, and the values obtained rose up from 3.03 MPa and 1.49 MPa for PVA-6-3 SN hydrogel to 25.15 MPa and 29.95 MPa for PVA-6-3/PEG-6-3 DN and PVA-6-3/PEG-4-3 hydrogels, respectively. Moreover, Zhang et al. [42] reported that mechanically strong DN hydrogels could be achieved by the combination of PVA and PEG through consistent cycles of freezing and thawing. It was announced that the compression strength increases when the number of freezing and thawing cycles increases, for example 0.015 MPa for PVA-6-1 SN hydrogel and 0.213 MPa for PVA-6-5 hydrogel. The influence of the repeating number of freezing and thawing cycles was more revealed for PVA/PEG hydrogels in comparison with pure PVA hydrogels. The compression stress-at-break increases from 0.087 MPa for PVA-6-1/PEG-4-1 DN hydrogel to 0.718 MPa for PVA-6-5/PEG-4-5 hydrogel. The same tendency was observed for the compression modulus values.

Peppas et al. [1] observed unusual formation of crystallite in aqueous solution of PVA during network consistent cycles of freezing and thawing. As proposed in the works of Osada et al. for PAMPS SN [29–34], the micro crystals in PVA hydrogels can also play a role as a physical crosslink and form three-dimensional network, which explain the extremely high mechanical stress-at-break. In the work of Zhang and collaborators [42], the crystal structure of PVA hydrogel was considered as double-layered structure held together by weak van der Waals forces operating between hydroxyl bonds, while the existing intramolecular hydrogen bonds were less of a reason for crystallinity to appear. It was suggested that above a certain, critical concentration, aqueous solutions of PVA and PEG could be separated in two phases, as follows: a) – condensed phase with high concentration of PVA and b) – dilute phase with high concentration of PEG. To explain the mechanical strength of PVA/PEG DN hydrogels Zhang et al. [42] proposed a model in which the high crystallisation appeared in the PVA condense phase, while dilute PEG phase crystallized between the cavities and the voids of the PVA. Additionally, between PVA and PEG the existence of entanglement was observed.

PVA SN is rigid, but brittle and heterogeneous, because of the voids generated during the consistent cycles of freezing and thawing. A part of poor crosslinked PEG SN is interpenetrated with the PVA, while the rest gets in the cavities where the energy is dissipated. As a result, partial entanglement of PEG with PVA SN can absorb the crack energy which occurs because of viscous dissipation or large deformation of its chains. The energy relaxes through the movement of the molecular chains which prevents the growing of the cracks to a macroscopic level and explains the mechanical strength of the PVA/PEG DN hydrogels [42]. However, when the crosslinking density of the second network is increased, the mechanical properties of the final DN decline.

The mechanical properties of PEU-XXX-YY SN hydrogel and PEU-XXX-YY-PMMA-Z interpenetrating polymer networks (IPNs) under compression behaviour were shown by Rakovsky et al. [43]. High values for compression modulus of hydrogels were achieved when the molecular weight of PEG was kept low, while the content of DEGDVE and PMMA were high. When the number of the average molecular weight between the crosslinks (M_c) of the PEU SN was high, an addition of 10% PMMA resulted in a little change of the modulus, and contrariwise. For example, PEU-2000-YY SN and PEU-
2000-YY-PMMA-10 IPN have values of 2.3 MPa and 2.7 MPa, respectively, while for PEU-1000-YY SN and PEU-1000-YY-PMMA-20 IPN the compression modulus increased significantly to 3.3 MPa and 5.75 MPa. The measured compression strength and strain-at-break for PEU-1500-0.5 SN and PEU-2000-0.75 SN was 1.5±0.5 MPa and ~55 % and 1.6±0.15 MPa and ~50 %, respectively. IPN of PEU-1500-0.5-PMMA-10 sustains a stress of 3.5±1.1 MPa and elongation-at-break of ~80 %, while PEU-2000-0.75-PMMA-10 broke down at 3.2±0.5 MPa and ~60 %.

The mechanical properties of kinds of triple network gels (or DN gels improved by molecular stent, respectively) were impressed through tensile and compression results in the Nakajima et al. work [44]. For the variety of components used, the highest value for tensile fracture stress with 1.95 MPa was obtained for PDMAAm (0.7-3-0.1) as first network, PAMPS (1-0.1) as molecular stent and PAAm (2-0.02-0.01) as second network, whereas in brackets the monomer concentration as well as mol percent of cross-linker and initiator, respectively, were specified.

In the Haque et al. work [45], the tensile experiments of PDGI/PAAm gels showed parallel to PDGI layer a stress-at-break of 600 kPa and in orthogonal direction only 38 kPa. However, the uniqueness of layer-structured hydrogels was mainly focused on their anisotropy and alterations of colors evoked by strain and compression tests.

As described in the Shin et al. work [46], biocompatible PGGMA/PGelMA DN hydrogels exhibit distinctive compression strength of nearly 7 MPa and failure strain of around 80 %. Furthermore, the DN hydrogel could act like a scaffold for fibroblast cells that are encapsulated in the DN matrix.

MECHANICAL PROPERTIES OF NANO- AND MICRO-PARTICLE REINFORCED COMPOSITE HYDROGELS

The mechanical properties of nano-particle reinforced composite hydrogels are listed in Table 5. In the works [47–49, 52] the compression and tensile measurements were performed on as-prepared hydrogels or swollen ones in definite water content [54]. In the works [50, 52] the hydrogel were tested at their EWC. Tensile and compression tests were executed in order to evaluate the mechanical properties of S_m and G_n gels [47]. As a result, the tensile stress-at-break values and Young’s moduli obtained for S-gels with different clay contents changed from 0.07 (S2) to 1 MPa (S15) and 0.05 to 0.074 MPa, respectively. The strain-at-break altered between 1304 (S10) and 1424 % (S12.5). In comparison, G-gels sustained a tensile stress of 0.074 MPa and reached a Young’s modulus of 0.05 MPa, while the elongation at breaking point went up to 1445 %.

The compression test was performed only for S-gels. For example, S15 gel reached in the strain range of 0–75 % only a compressive strength of nearly 2 MPa, while a significant rising up to 20 MPa was observed between 75–92 %. However, the gel was not damaged during the compression test, and after release, it recovered to about 50 % of its original size at ambient temperature. To explain the mechanical properties of S gel under compression, Liu et al. [47] proposed two reasons: a. polymer chain segments show a high
Table 5. Mechanical results and measuring parameters of nano-particle reinforced hydrogels

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Testing Method</th>
<th>Set of parameters</th>
<th>Results</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Stress-at-break $\sigma$ [MPa]</td>
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<tr>
<td>[47]</td>
<td>a) Tensile and b) Compression</td>
<td>a) $d = 5$, $L = 80$, $L_1 = 30$, $v_2 = 100$ b) $a = 8.5$, $b = 8.5$, $s = 11$, $v = 5$ %/min</td>
<td>a) 0.07–1 b) 20</td>
</tr>
<tr>
<td>[48]</td>
<td>a) Tensile and b) Compression</td>
<td>a) $d = 5.5$, $L = 70$, $L_1 = 30$, $v_2 = 100$ b) $a = 10$, $b = 10$, $s = 10$, $v_1 = 0.5$</td>
<td>a) 0.1–3 b) 0.7–5</td>
</tr>
<tr>
<td>[52]</td>
<td>Compression</td>
<td>$d = 6$, $L = 12$, $v_2 = 1$  ($ASTM$ D695)</td>
<td>8.5±2.0–19±2.0</td>
</tr>
<tr>
<td>[53]</td>
<td>Compression</td>
<td>$a = 8$, $b = 8$, $s = 6$, $v_2 = 0.1$ mm/s</td>
<td>~1.6 2)</td>
</tr>
<tr>
<td>[54]</td>
<td>Compression</td>
<td>$d = 24–26$, $s = 12–18$, $v_2 = 5$</td>
<td>0.8–78.6 3) 1.4–7.9 4)</td>
</tr>
<tr>
<td>[55]</td>
<td>a) Tensile and b) Compression</td>
<td>a) $d = 5$, $L = 100$, $L_1 = 50$ b) $d = 50$, $s = 30$, $v_2 = 20$</td>
<td>a) 0.193–0.266 b) 1.88–4.6</td>
</tr>
<tr>
<td>[56]</td>
<td>Compression</td>
<td>$d = 8$, $s = 6$, $v_1 = 25$ µm/s</td>
<td>~0.012–~0.055 5)</td>
</tr>
<tr>
<td>[57]</td>
<td>Compression</td>
<td>$d = 14$, $s = 8$, $v = 2$ mm/min</td>
<td>1.41±0.04– 8.44±0.13</td>
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<td></td>
<td></td>
<td></td>
<td>a) 0.0809±0.0372–0.2195±0.0705</td>
</tr>
<tr>
<td>[58]</td>
<td>a) Tensile and b) Compression</td>
<td>a) $L = 25$, $b = 1.6$, $s = 1.3$, $v = 20$ b) $d = 10$, $s = 7$, $v_1 = 10$</td>
<td>b) 0.3961±0.0464–3.7287±0.1541</td>
</tr>
<tr>
<td>[59]</td>
<td>Tensile</td>
<td>$v_2 = 10$  ($Standard$ ASTM D638-09)</td>
<td>3.9±0.9– 7.2±0.2</td>
</tr>
<tr>
<td>[60]</td>
<td>Tensile</td>
<td>$L = 30$, $b = 10$, $s = 1$, $v_2 = 100$</td>
<td>~0.05–0.56</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>a) 0.10±0.02–0.798±0.223</td>
</tr>
<tr>
<td>[61]</td>
<td>a) Tensile and b) Compression</td>
<td>a) $L = 5$, $b = 3$, $s = 1$, $v = 5$ mm/s b) $d = 5$, $s = 10$, $v_2 = 0.01$ mm/s</td>
<td>b) 0.048±0.004–0.066±0.005 6)</td>
</tr>
<tr>
<td>[62]</td>
<td>Tensile</td>
<td>$L = 10$, $b = 5$, $s = -$, $v = 5$</td>
<td>1.6</td>
</tr>
<tr>
<td>[63]</td>
<td>Tensile</td>
<td>Cylindrical-shaped</td>
<td>0.016–0.85</td>
</tr>
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</table>

1) Tensile strength measured in a second cycle
2) Compression strength measured at 32.5 °C
3) Compression strength for MMC (A1-A6) hydrogels
4) Compression strength for MMC (B1-B4) hydrogels
5) Nominal stress
6) Compression stress at 50 % strain

ability to move in water, and therefore, an elastic state can be assumed for the polymer, and b. mono-modal molecular weight distribution of polymer chains were created between
the crosslinks, caused by homogeneous dispersion of clays in water and the significantly lower crosslinking density in comparison with gels crosslinked by MBAA.

In the works [48–51] the results from tensile and compression tests for NC1–NC25 gels are reported. Different tensile stress-strain profiles were obtained for NC gels elongated as prepared then remained to recover for one hour and again elongated. In the first cycle, the tensile strength and strain varied between 0.1–1.1 MPa and 1250–900 %, respectively. The Young’s modulus for both cycles was evaluated for elongation between 10–50 % (E_{10–50}) and 100–200 % (E_{100–200}). The highest values for E_{10–50} and E_{100–200} were achieved to be ~0.6 MPa and ~0.1 MPa (both for NC25 gel). In the second cycle, the tensile strength and strain altered between 0.1–3 MPa and 1100–150 %, respectively. The maximum values obtained for E_{10–50} and E_{100–200} were similar, and in fact around 1 MPa.

In the compression stress-strain curve of NC gels, an effect of linear slope of the stress at low strain was observed, followed by a rapid increase at high strains. The compressive strength increased with higher clay contents in the gel and changed from 0.7 to 5 MPa, respectively, while the obtained strain was around 80 % in all cases. The compressive modulus follows the same tendency like the compressive strength. The obtained values altered between 0.025–0.45 MPa and were calculated for low strains (10–17 %).

To explain the mechanical properties of NC gels, Haraguchi et al. [48] proposed two structural models (NC gels with C_{clay} < 10 and C_{clay} > 10). In both cases, three different structures were proposed, and in fact a) as-prepared, b) elongated and c) recovered state, consequently. In NC gels with low clay content (C_{clay} < 10), the clay pellet was orientated in direction perpendicular to the stretch orientation, as reported by the SANS study [48]. For NC gels with high clay content (C_{clay} > 10), it was estimated from the changes in the mechanical properties that the clay pellet probably was put parallel to the elongated direction. Moreover, the high clay concentration in combination with their arrangement to direction of elongation, the orientation and the strain was occasionally retained even after long relaxation time. This caused the enhanced mechanical properties which were obtained in the second cycle.

In a work [52], the compressive strength, maximum deformation and elastic modulus of HYAFF11/α-TCP-x composite hydrogels were presented. Here, the stress-strain profile of HYAFF11/α-TCP(7/93) and HYAFF11/α-TCP(14/86) gels immersed in water are compared at different reaction times. It was found that the compressive strength for both composite hydrogels increases in a linear relation following a function of water immersion time, while the elastic modulus rises as the reaction proceeds. However, the compression strain-at-break decreased because of the hardening of the structure. The compression stress-at-break and compression modulus for HYAFF11/α-TCP(7/93) gels varied between 8 MPa (in case of no reaction), 11 MPa (24 hours reaction time) and 190–380 MPa (96 hours reaction time), respectively. For HYAFF11/α-TCP(14/86) gels was found as follows: 12.0 MPa (no reaction), 19 MPa (24 h) and 150±40 (no reaction) – 260±30 MPa (96 hours), correspondingly. The compression strain-at-break for HYAFF11/α-TCP(7/93) gels changed between 5–9 %, while for HYAFF11/α-TCP(14/86) gels, this value varied from 12–26 %. The preparation of HYAFF11/α-TCP happened after the extraction of the polymer solvent with ethanol.
whereby the α-TCP particles were inserted in the three-dimensional structure of HYAFF11. In the presence of water or biological fluids, the α-TCP hydrolyses was obtained by dissolution reaction, followed by precipitation of calcium deficient hydroxyl-apatite (CDHA) needle-formed crystals. As a result, an entangled network was built.

Compression stress-strain curves of Polyurethane/PNIPA gel composites were presented by Liu et al. [53]. The maximum compression stress and strain measured at 32.5 °C were ~1.6 MPa and 12 %, respectively. The values measured for elastic modulus were different at different temperatures and changed from 0.0128 MPa at 25 °C to 0.458 MPa at 40 °C. In comparison with the Polyurethane/PNIPA gel composite, the compression strength and strain-at-break for pure PNIPA gel at 35 °C were obtained to be 0.3 MPa and 1.2 %, consequently. The compression moduli differed from 0.0069 MPa at 25 °C to 1.847 MPa at 40 °C. The compression moduli of the gel composites were significantly lower unlike the pure gel at high temperatures and higher than the pure gel at low temperatures. Using the reinforcement strategy, Liu and collaborators [53] explained the achieved higher stiffness of the polyurethane/PNIPA gel composite in the swollen state. It was also reported that in comparison with the pure PNIPA gel which can be broken easily by handling, the gel composites are much stronger.

Furthermore, in the work [54], the compressive behaviour of a series of MMC (A1-A6) and MMC (B1-B4) hydrogels were measured. The MMC (A6) hydrogel did not break even at a stress of 10.2 MPa and strain of 97.9 %. Cyclic compression tests up to 90 % strain were performed for MMC (A2) hydrogel, but only a little change in the stress-strain profile was observed which supposes that no irreversible damage in the hydrogel occurred. The MMC (A1-A4 and A6) hydrogels with high water content (89 wt. %) recovered nearly to their original shapes after the compression test, while the MMC hydrogels (A4-A5) with lower water content fractured under high stresses and strains of 78.6 MPa and 99.3 % for MMC (A4) as well as 66.7 MPa and 95.5 % for MMC (A5), respectively. The compression stress and strain for MMC hydrogels increased with increasing the monomer concentration (A series) from 0.8 (A1) to 78.6 MPa and 95.5 (A5) – 99.3 %, respectively and reaction time for B series as follows: 1.4 (B3) – 7.9 (B4) MPa and 98.4 (B2-B3) – 99.7 (B1, B4) %, but decreased with increasing of the MMS concentration. The stress at 96 % strain as well as elastic modulus increased when the water content in the gel was decreased. The low values, obtained for the compression modulus of MMC hydrogel composite could be explained with the existence of long flexible chains between the crosslinker (MMS) as can be proposed from the classic rubber elasticity theory. An increase of the strain was a reason for some chains between the crosslinker to come close to their full extension which caused enhance in the stress. It was reported that the water content in the hydrogel influenced the hydrogen bonds and the effectiveness of the entanglement of the chains, respectively.

The tension experiments as well as compression stress-strain measurements were performed on HRM hydrogels [55]. It was reported that the tensile strength reached was 0.193 (HRM0.9-2.0) – 0.266 (HRM0.9-2.0) MPa and elongation of 323 (HRM2.4-2.5) – 553 (HRM3.0-2.5) %. During the compression test, HRM hydrogels did not break even at 90 % strain and sustain a high stresses between 1.88 (HRM3.0-2.5) – 4.6 (HRM2.4-2.5) MPa.
The compression test measurement was also performed on HM. It was found [55] that at 0.85 MPa stress and 69 % strain HM fractured. It was reported that HRM act like multifunctional crosslinking agents, which crosslinked the linear chains coming from AAm and AMPS monomer at the time of polymerization. Unlike conventional crosslinking agents, the polymer chains between two neighbouring HRM microspheres are long, coiled and flexible with narrow distribution. Because of this, the stresses can be dissipated by the long flexible chains crosslinked on HRM microspheres. As a result, the obtained HRM hydrogel had significantly higher mechanical strength in comparison with the conventional hydrogels.

Lubricated, uniaxial compression tests were performed on cylindrical-shaped Si(y) hydrogels samples in EWC conditions [56]. Through the Mooney concept, the non-linear character of hydrogel materials at significant deformations was investigated. Moreover, the reduced stress ($\sigma_{\text{red}}$, calculated by $\sigma_{\text{red}} = \sigma_{\text{nominal}}/(\lambda - 1/\lambda^2)$) against the inversed of the extension ratio (1/\lambda) was also designed. The $\sigma_{\text{red}}$ presents information about strain-dependent shear modulus $\mu$. In the case of the negligible value of 1/\lambda, the $\sigma_{\text{red}}$ and the shear modulus determined in the linear region of the curve are comparable. However, if the $\sigma_{\text{red}}$ possesses a constant as a function of \lambda, the classic rubber elasticity concept is gained. The behaviour described is for the case of a pure PDMA sample which shows a strain enhancing effect at significant strains. Further, an increase of the filler concentration caused considerable expression of the strain hardening effect. Lin et al. [56] explicated the molecular reason for the strain hardening by the changes from entropic to the enthalpy elasticity of the polymer chains in completely extended state. In the case of high cross-link and homogeneous polymer system, the strain hardening is extremely sharp. However, rises of the filler concentration led to strain hardening at reduced strains. The greatest values of $\sigma_{\text{red}}$ and 1/\lambda were obtained for the Si100 hydrogel ~0.46 MPa and ~2.75, whereas the slightest were reached for Si25 hydrogel and in fact ~0.05 MPa and 2.0.

The hysteresis behaviour of PDMA and PDMA-silica hybrid hydrogels was further evaluated. Contrary to the PDMA specimen which presented no hysteresis, the loading-unloading curves of hybrid materials diverged from each other which propose the presence of dissipation. Lin et al. [56] assessed the existence of permanent fracture over the time scale of repeating loading-unloading tests and the dependency among the amplitude of the hysteresis as well as the maximum strain throughout the loading stage. In consequence by series of hysteresis cycles performed on the same sample at decrease quantity of $\lambda_{\text{max}}$, it was demonstrated that all loading curves fell on the same master curve, which gave clear evidence that the structure of the hydrogel returns to the original state after each deformation cycle. However, the amount of energy dissipated at the time of the cycle depended on the achieved $\lambda_{\text{max}}$. The described effect designated a complete absence of the Mullins effect, i.e. a permanent or very gentle relaxation in the structure after the first hysteresis cycle (contrast to DN hydrogels).

The mechanical properties of CH and TPH hydrogels were determined by compression experiments [57]. Despite contents of 90 wt. % water, the TPH3 hydrogel (NIPAM/SN/Initiator/Water = 1/0.75/0.005/9.0) sustains a strength of 6.9 MPa and fracture strain of 92.8 %, which is significantly greater in comparison to the CH hydrogel ~0.03 MPa and
Moreover, the compression properties of TPH hydrogels synthesized with different SN contents are compared. In consequence, an increase in the SN quantity caused an enhancement in stress-at-break, e.g. the greatest fracture stress – 8.44 MPa was achieved for TPH4 hydrogel (NIPAM/SN/Initiator/Water = 1/1.0/0.005/9.0), although the further rise in the amount of SN affected its reduction. Tan et al. [57] attributed the decline of the mechanical properties to the possible aggregation of SNs which acts as defects in place of a crosslinker. The compression strain-at-break of TPH hydrogels demonstrated a decreasing trend due to the increase of the cross-linking density.

The effect of Laponite XLS nanoparticles on the mechanical properties of PEGDA\textsubscript{x}NC\textsubscript{y} hydrogels were investigated by compressive and tensile test measurements [58]. The compression performances of equilibrium swollen (in PBS) PEGDA\textsubscript{10K}NC\textsubscript{y} hydrogels (y = 0, 2.5, 5 and 10 %) demonstrated a concentration-dependent enhancement. In comparison to the PEGDA\textsubscript{10K}, the PEGDA\textsubscript{10K}NC\textsubscript{10} hydrogel exhibited an extensively improved stress-at-break (0.142±0.1049 vs. 3.728±0.1541 MPa), strain-at-break (68.8±11.0 vs. 94.9±2.4 %) and compression modulus (0.0244±0.0043 vs. 0.0379±0.0107 MPa).

The tensile properties of PEGDA\textsubscript{x}NC\textsubscript{y} (x = 3.4 and 10K, y = 0 and 10 %) in equilibrium conditions were also compared. The slightest tensile stress and Young’s modulus (0.0302±0.0197 MPa and 0.0262±0.01 MPa) were determined in the case of PEGDA\textsubscript{10K}, although the lowest fracture strain (72.1±2.9 %) was obtained for PEGDA\textsubscript{3.4K} hydrogel.

The reported results designated PEGDA\textsubscript{x}NC\textsubscript{y} hydrogels as extreme resistant materials versus compressive and tensile forces. In contrast to conventional hydrogels, the enhanced mechanical properties of PEGDA\textsubscript{x}NC\textsubscript{y} could be assigned to the energy dissipating effect of rigid Laponite XLS nanoparticles as well as interaction among Laponite XLS nanoparticles and PEGDA polymer chains. It was demonstrated that the effect of Laponite XLS nanoparticles on the mechanical properties changed by the M\textsubscript{w} of PEGDA (for instance the mechanical characteristics of PEGDA\textsubscript{10K}) were improved versus the PEGDA\textsubscript{3.4K} hydrogel. Chang et al. ascribed to the M\textsubscript{w} a dependent increase in mechanical properties by significant interactions among high M\textsubscript{w} PEGDA and Laponite XLS nanoparticles. However in the case of PEGDA\textsubscript{3.4K}NC\textsubscript{y} hydrogels, the mechanical characteristics were determined by the chemical cross-linked network due to the low M\textsubscript{w} among the cross-link points (M\textsubscript{c}). Consequently, the hydrogel systems possess negligible M\textsubscript{c} and demonstrate more seldom places of interactions caused by Laponite XLS nanoparticles. Therefore, they do not present a significant effect on overall mechanical properties.

Wang et al. explored the mechanical properties of BC/PVA hydrogel composites with different BC contents – 12, 17 and 27 % [59]. All measurements were performed in Krebs solution at 37 °C. An incorporation of BC caused an increase in the tensile strength and Young’s modulus, and in fact 3.9, 5.8 and 7.2 MPa as well as 24.0, 38.7 and 63.0 MPa, respectively. The enhanced mechanical properties of BC/PVA hydrogels were assigned to the extreme stress-at-break and tensile modulus of BC nanofibrils and generation of hydrogen bonds among PVA and hydroxyl groups of BC. In contrast, the elongation at break decreased by the introduction of BC, for instance ~380, 155, 100 and 69 % at 0, 12, 17 and 27 % BC. Wang et al. [59] described the converse influence by a specific arrangement
of BC nanofibrils instigated through the formation of hydrogen bonds. Consequently, the effect of the force caused changes in the fibrils orientation and declined the strain-at-break.

The tensile stress-strain behaviour of tetra-PEG\textsubscript{ph} and diverse tP-NC2\textsubscript{ph} hydrogels (\(c_p = 120 \text{ mg/mL}\)) were compared \[60\]. The tensile properties of tP-NC2\textsubscript{ph} were comparable to the tetra-PEG gel. In the case of tP-NC2\textsubscript{ph}-N and tP-NC2\textsubscript{ph}-A gels, the stress-at-breaks were analogous to the tetra-PEG gel \(\sim 0.09 \text{ MPa}\) although the Young’s modulus was non-considerable distinctively: tP-NC2\textsubscript{ph}-N > tP-NC2\textsubscript{ph}-A > tP-NC2\textsubscript{ph}-AN. The obtained results indicated higher interaction among clays and TN-PEG than between clays and TA-PEG. The decrease of tensile modulus and strength was ascribed to the significant higher viscosity caused by the difficulty at time of mixing. Moreover, an increase in the \(c_{\text{clay}}\) led to the decrease in the mechanical properties of hydrogels. The tensile performances of tP-NC2\textsubscript{py}-N, tP-NC2\textsubscript{py}-A and tP-NC2\textsubscript{py}-AN gels were determined. The greatest values for tensile modulus, stress-at-break and strain-at-break were obtained for tP-NC2\textsubscript{py}-A gel. The adding of clays to the TN-PEG solution was used to prepare tP-NC2\textsubscript{py}-N and tP-NC2\textsubscript{py}-AN gels which possess extremely high mechanical properties, although it is not suitable for the synthesis of tP-NC hydrogels. In comparison to the tetra-PEG\textsubscript{py} gel, the Young’s moduli of tP-NC2\textsubscript{py}-N and tP-NC2\textsubscript{py}-AN gels were considerably reduced. The durable interactions among clay and TN-PEG molecules in solution intervene in the polymerization of TA-PEG and TN-PEG. Consequently, the tensile modulus of tetra-PEG gel was significantly higher, related to the tP-NC2\textsubscript{py}-N and tP-NC2\textsubscript{py}-AN gels.

The influence of clay content and polymer concentration on the mechanical properties of tP-NC hydrogels was considered. A sequence of identical and clear tP-NC\textsubscript{n}-A gels with different \(c_{\text{clay}}\) \((n = 1–20)\) and steady \(c_p\) \((120 \text{ mg/mL})\) was prepared by aid of pyrophosphate-Na buffer solutions. An increase of \(c_{\text{clay}}\) in the range of NC0 to NC2 caused considerably enhance in the tensile strength and elongation at break, for instance \(\sim 0.08 \text{ MPa} \) \((\text{NC0}) – 0.3 \text{ MPa (NC2)}\) and \(\sim 490 \% \) \((\text{NC0}) – \sim 900 \% \) \((\text{NC20})\). Further, a raise of \(c_{\text{clay}}\) beyond NC2 elicited a decrease in the stress-at-break but subsequently a negligible decline and an enhancement of strain-at-break. The tensile moduli demonstrated analogous dependence of \(c_{\text{clay}}\) and were determined by the slopes between 10–50 \% and 100–200 \% elongation – \(E_{10–50}\) and \(E_{100–200}\). For instance, an increase of \(c_{\text{clay}}\) between NC0 – NC2 caused the rising to \(E_{10–50}\) and \(E_{100–200}\) however an additional increasing provoked the decrease. The results revealed that negligible quantities of clay \((c_{\text{clay}} \leq 2 \times 10^{-2} \text{ mol/L})\) can be successfully incorporated inside the tetra-PEG and act as cross-linker to improve the mechanical properties. Nevertheless, further rise of \(c_{\text{clay}}\) caused a decline in the Young’s moduli and tensile strength, respectively, because the construction of tetra-PEG is disrupted by the higher amount of clay platelets.

To expose the effect of the network density on the mechanical characteristics of tP-NC hydrogels, distinctive categories of tP-NC\textsubscript{\textsubscript{n}}\textsubscript{py}-A gels were prepared, whereas each contained macromonomer represented by different \(M_w\) \((5K \text{ and } 20K)\). By increasing \(c_{\text{clay}}\), the \(E_{10–50}\) of tP-NC\textsubscript{n}\textsubscript{py}-A \((5K)\) gel gained a highest value at NC8, although in the case of tP-NC\textsubscript{n}\textsubscript{py}-A \((20K)\) gel the maximum was determined at NC2. The obtained results designated that the \(M_w\) of macromonomer was responsible for the amount of clay that can be
successfully introduced in tP-NC gel. In consequence, low M₀ macromonomers are able to create a regular network structure although at short interclay distances.

The influence of cₚ (80–240 mg/mL) on the tensile properties of tetra-PEG and tP-NC₂py-A gels was determined. In each case, an increase in the cₚ caused an enhancement in the Young’s moduli and stress-at-break, respectively. The best tensile strength and elongation at break were achieved for tP-NC₂py-A gel (180 mg/mL), and in fact 0.56 MPa and 1000 %.

An original synthetic technique to prepare tP-NC gels having exceptional optical and mechanical properties was established in the literature. Fukasawa et al. reported that the use of pyrophosphate-Na buffer and the combination of clay suspension by TA-PEG macromonomer were the most proper conditions to obtain tP-NC gel with excellent properties. By instrumental analysis and mechanical experiments of tetra-PEG/Clay structure, it was demonstrated that the clay platelets were consistently dispersed and steadily incorporated inside the tetra-PEG system due to possible interactions among them (hydrogen bonds).

The mechanical properties – tensile and compression test measurements of as prepared PEG-nHAp hydrogels were evaluated at ambient temperature [61]. An increase in the concentration of nHAp – 0–15 % caused a significant enhancement in the stress-at-break (0.1–0.8 MPa) and tensile modulus (0.004–0.015 MPa), respectively. Gaharwar et al. attributed the improved mechanical performance to the presence and distribution of nanoparticles inside the polymer network. Despite the creation of aggregates at substantial nHAp concentration – 15 %, no adverse influence on the mechanical properties was established.

The elongation at break of covalently cross-linked PEG hydrogel was negligible in comparison to polymer materials strengthened by nHAp. An increase in the concentration of nHAp (5, 10 and 15 %) instigated an improvement in the strains – 1743±111 %, 1905±167 % and 1970±169 %, correspondingly. It was supposed that the presence of covalent cross-links restricts the chain movements inside the PEG SN hydrogel whereas the attendance of physical cross-links among PEG chains and nHAp enable the chain motions. The significant elongation and toughness of polymer nanocomposite ascribed the authors because of the combination of polymer-polymer and polymer-nanoparticle interactions that intervene by the permanent cross-links of PEG at the time of polymerization.

In the case of insignificant strains ~200 %, PEG-nHAp hydrogels were considerable elastic and restored to the original structure after the stress was released. Conversely, an increase of the strain caused non-complete recovery of the nanocomposite system through the fracture of covalently cross-linked PEG SN, but the presence of interpenetrating physical networks is able to partially compensate the rupture of covalent bonds. The physical interactions among the network-bounded polymer chains and nanoparticles were reversible and probably represented by ionic/hydrogen bonds. However, the exact interactions were indistinct and required supplementary investigations.

The compression properties of PEG SN and PEG-nHAp NC hydrogels were determined. All hydrogel materials demonstrated large elastic behaviour and returned back to the initial state after deformation was released. The hysteresis loading-unloading cycles of PEG SN hydrogel overlapped each other although the introduction of nHAp, represented by
diverse concentration caused steady increase of energy loss at the time of the experiment. Continual cycles of loading-unloading tests resulted in a similar tendency.

An incorporation of nanoparticles instigated an enhancement in compression moduli and stress-at-break at 50% strain, respectively. Approximately a double increase in the moduli was achieved when the concentration of nanoparticles changed from 0 to 15%, for instance 0.0482±0.0129–0.0825±0.0037 MPa, although the compression strength enhanced slightly – 0.0506±0.0025–0.0664±0.0048 MPa. Moreover, in the case of the experiments that were performed at diverse strain rate negligible differences in the compression moduli were observed. The combination of bioactive properties of nHAp and the elastomeric properties of PEG hydrogels, injectable and bioactive templates can be developed explicitly for orthopaedic tissue regeneration.

The PNIPAM-nanoclay (L-NC) gels were measured in tensile experiments and due to the layered and interconnected structure they could achieve high stress-at-break and strain-at-break values of 1.6 MPa and 740%, respectively [62]. These above average results at clay content of 23% are explained through hydrogen bonding between stacked clay sheets and polymer chains. In contrast, a random R-NC gel without prior vacuum filtration obtained a 5 times lower stress-at-break at highest as possible clay content of 10%.

The nanoclay-reinforced hydrogels prepared through PNIPAM and SiPN exhibited the thermo responsive effect with change in volume due to alteration in the hydrophilicity of polymer chains [63]. The tensile testing of cylindrical-shaped specimens has shown in equilibrium swollen state only poor stress-at-break results of 18 and 16 kPa for PNIPAM and SiPN, respectively. However, the as-prepared hydrogel achieved much higher values of 85 and 63 kPa for PNIPA and SiPN. Similar findings could be observed for strain-at-break where the hydrogels in equilibrium state reached values of 150 to 240% while the as-prepared material sustained elongations from 600 to 900%. Furthermore, repeated loading-unloading experiments until 200% elongation exhibited a hysteresis behaviour and interestingly also a Mullins effect.

MECHANICAL PROPERTIES OF MISCELLANEOUS HYDROGELS

In Table 6, the mechanical properties of group “miscellaneous” hydrogels are summarized. To measure the mechanical properties of HEMA-stat-MBC hydrogels, a series of tensile test measurements were performed [64]. The tensile strength of poly(HEMA) hydrogel was 0.35 MPa, while it was improved for HEMA-stat-MBC hydrogels significantly when relatively low quantities of MBC were introduced, for example from ~0.8 MPa (HEMA/MBC = 95:5) to 1.3 MPa (HEMA/MBC = 85:15). The tensile strain-at-break was 100% for pure poly(HEMA) hydrogel, while for HEMA-stat-MBC hydrogels altered between ~80% (HEMA/MBC = 90:10) and 110% (HEMA/MBC = 95:5 and 85:15, respectively).

The tensile properties of hydrogels obtained by Click chemistry were evaluated and supplementary compared to the performances of PEGDA hydrogels prepared by photo polymerization [65]. An increase of the $M_n$ of PEG precursors (3.4–10 kDa) correlates to greater pore sizes, respectively raising the EWC and declining the Young’s modulus. In
comparison to the photo polymerized PEGDA hydrogels, Click hydrogels demonstrate significant stress-at-break and strain-at-break, for example 0.07 MPa and 150 % (M_n = 14 kDa) versus 2.39 MPa and 1550 % (M_n = 10 kDa). Malkoch et al. [65] ascribed the extreme mechanical properties of Click hydrogels to the structured character of the cross-linking reaction caused by the extra dispersion of crosslinks points. The possibility to change the structure and respectively the mechanical performance of hydrogels in a controlled manner is a critical aspect in the field of biomedical applications.

Bio-conjugation of P(NIPAAm[A]-co-AAc[B]-co-NAS[C]-co-HEMAPLA[D] hydrogels with different amounts of collagen (5 and 10 %) were made, and tensile tests for hydrogels with and without collagen were executed [66]. Hydrogels without collagen sustained stresses higher than 0.5 MPa, elongations-at-break higher than 1330 % and Young’s moduli surpassed 0.05 MPa. It was found that decreasing the ratio between NIPAAm/HEMAPLA from 85:4 to 80:9 enhanced significantly the tensile strength (0.5 MPa for NIPAAm/HEMAPLA = 85:4 and ~0.9 MPa for NIPAAm/HEMAPLA = 80:9), while a further decrease to 75:14 reduces the tensile stress-at-break to ~0.5 MPa. The elongation-at-break and Young’s modulus increased when the NIPAAm/HEMAPLA ratio was decreased and changed to 1398 % and 0.05 MPa for NIPAAm/HEMAPLA = 85:4 as well as to 1842 % and 0.12 MPa for NIPAAm/HEMAPLA = 75:14).

The amount of AA in the hydrogels as well as lactate units also affected the mechanical properties of the hydrogels. An increase of the AA content from 6 to 11 % decreased the tensile strength and Young’s modulus from 0.6 MPa (NIPAAm/AA/NAS/HEMAPLA = 85:6:5:4) to 0.55 MPa (85:11:5:4) and 0.53 MPa to 0.046 MPa, respectively, while the elongation-at-break increased in the same order from 1398±87 % to 1580±138 %.

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<th>Table 6. Mechanical results and measuring parameters of “other” hydrogels</th>
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\(^{1}\) Values contributed to CW
number of lactate units added into the hydrogels (NIPAAm/AA/NAS/HEMAPLA = 85:6:5:4) increased the tensile stress-at-break and modulus up to 0.6 MPa and 0.53 MPa, respectively (for 2.1 lactate units) and to 1.1 MPa and 0.117 MPa (for 3.9 lactate units), respectively. However, the tensile strain decreased from 1398 to 1196 %.

An addition of 5 or 10 % collagen into the hydrogel reduced the tensile strength and strain-at-break to ~0.3 MPa and 344 %, while the Young’s modulus was not affected.

The mechanical properties of P(NIPAAm[A]-co-AAc[B]-co-NAS[C]-co-HEMAPLA[D]) hydrogels changed in agreement with HEMAPLA content, PLA length, AA content and incorporating of collagen. An increase of HEMAPLA content or PLA length decreased the hydrophilicity and the water content in the hydrogel, respectively, which could enhance the intermolecular interactions in the copolymer and in this way to increase the tensile strength. Rising amounts of AA content or incorporating collagen into the hydrogels elicited an increase of the water content and a decrease of the interactions in the copolymer, which declined the mechanical strength.

The mechanical properties of Chitosan/PEG/PNIPAAm (CGN#) hydrogels were measured using tensile tests [67]. When M<sub>W</sub> of PEG increased from 2000 to 6000 g/mol, the tensile strength and strain increased from 36 to 40 MPa and from 22 to 41 %, respectively. The further changing of M<sub>W</sub> of PEG from 6000 to 20000 g/mol declined the mechanical properties from 40 to 25 MPa and from 41 to 34 %.

Level of crystallinity and intermolecular forces in CGN hydrogels are two factors proposed from Sun et al. [67] to explain the mechanical properties. It was reported that CGN2000 hydrogel has high crystallinity but short chains, and as a result, the intermolecular forces are relatively weak because of minor entanglement between PEG and Chitosan/PNIPAAm. When the M<sub>W</sub> of PEG grew up to 6000 g/mol, intermolecular forces were higher due to an increasing entanglement. While the effect of crystallinity remained relatively unchanged, it resulted in an enhancement of the mechanical strength. Further increase of M<sub>W</sub> caused a decline in the tensile stress-at-break because the effect of crystallinity of the CGN hydrogels was reduced significantly, and the hydrogels lacked higher intermolecular forces to support the mechanical properties.

The strain-at-break of Chitosan/PEG/PNIPAAm hydrogel altered in the same way as the tensile strength, when the M<sub>W</sub> of PEG was increased because a lower crystallinity was reached. It was reported that the crystallinity acted as a physical crosslink which limited the elongation-at-break [67].

The tensile stress-strain profile of CP[X] and CW hydrogels was presented in Ref. [68]. The tensile strength of CP[X] hydrogels is much higher in comparison with this measured for CW hydrogels and changed between 3.5 (CP200) to 7.9 (CP800) and 1 MPa for CW, respectively. The tensile strain-at-break for CP[X] altered from 40 (CP1000) to 100 % (CP200) and reached for CW hydrogels nearly 80 %. The Young’s modulus of CP[X] increased linearly with growing up of M<sub>W</sub> of PEG and reached the value of 83.7 MPa.

Liang et al. [68] reported that microcrystal structures in the CW hydrogel were created by aggregations of inter- and intrachains of the cellulose. The combination of the hydrophilic effect of the cellulose chains accompanied by the microcrystal structure caused
weak interactions between cellulose chains and water molecules. As a result, the water molecules in the CW membrane effect as small molecules, which fill the voids created by the cellulose chains and played no role on the crystal structure of the cellulose. However, when the water was replaced by PEG, the structure of CP hydrogels significantly changed. It was reported that the cellulose and PEG chains in the gels can form a hydrogen bonds in between and as a result, the microcrystal structure of the cellulose was fully or partially broken by increasing the amorphous region in the CP gels. Due to the strong swelling effect of PEG, the interactions between the cellulose chains decreased, and they obtained a higher flexibility. Therefore, space for entanglement and re-aggregation of the cellulose during preparation of the hydrogels was obtained. As a result, homogeneous structure in CP gel was created, proved by the high mechanical strength.

The mechanical properties of Jf, JF-AA(AX), JF-AA(By), JF-AAm(AX) and JF-AAm(By) were compared [69]. Despite the high EWC of 99 %, the fracture compression/tensile stress and strain were significantly on an increased level in comparison to the conventional synthetic hydrogels, and in fact 1.16/0.0213 MPa and 80/160 %, respectively. The scanning electron microscopy (SEM) investigation of JF hydrogels designated the presence of layers structures, and a lot of fibers were attached to the layers of the pore walls. These layers represent extreme condensed and regular slices which are able to enclose many fibers. Wang et al. ascribed the great mechanical properties of JF systems to the structure stated: the layered structure causes a distribution of stress applied closely to the crack tip and ensures the requirement of specific external forces to affect a chain scission. Moreover, the effective interactions among the layers and fibers instigated the negligible strain-at-break and high modulus, respectively.

The compression and tensile properties of hybrid hydrogels were determined. In the case of JF-AA(B3) and JF-AA(B4) hydrogels, the compression strain-at-break extended beyond 90 %. However, the rest of the samples presented absence of rupture, although having strains higher than 95 %. An increase of the c_M caused a significant rise in the compression strain, whereas the increase of the c_C up to 0.20 % instigated a primarily an enhancement but subsequently a decline. The highest compression stress-at-break was obtained for the JF-AA(A3) hydrogels – 28.8 MPa at 95 % strain. The dependence of c_M and c_C on the tensile properties was analogously to the observed in compression experiments. The extreme tensile stress-at-break was determined in case of JF-AA(B3) hydrogel and in fact ~0.1 MPa.

The mechanical strength of JF-AAm hydrogels were significantly higher, compared to the JF-AA hydrogels [69]. The compression stress-at-break of JF-AAm(AX) hydrogels was 7–30 times higher than the JF hydrogel. The extreme mechanical strength of 34.9 MPa was obtained in the case of JF-AAm(A3) hydrogel. The supplement of a crosslinker caused an enhancement in compression stress-at-break and a decline in tensile stress-at-break, respectively. However, a significant amount of a crosslinker instigated the remarkable decrease in the compression strength. The JF-AAm hydrogels demonstrated analogous elongations as JF hydrogels.

The incorporation of a synthetic hydrogel inside the JF hydrogel by applying the radiation-induced polymerization and crosslinking process is an attractive approach to
create a hybrid hydrogel which possesses great mechanical performance. Wang et al. presumed strong interactions between the JF hydrogel and PAA, or respectively, PAAm hydrogel synthesized inside it. A very promising approach in addition to homopolymerization explained the reaction of monomers to associate with layers and fibers in order to induce covalent connections between both networks. The grafting reaction on to the JF hydrogel was probable due to the high-energy irradiation which instigated breakage of C-H or other bonds of the biomacromolecular chains, and therefore, the generation of macromolecular radicals was able to initiate the grafting process. As supplement to the covalent linking, the polymer network created inside the JF hydrogel was bound to the entanglement by the nanofibers of the JF hydrogel, and was able to generate hydrogen bonds with the biomacromolecules.

In the work [70], the tested PEG/PAA IPN hydrogels reached in compressive testing a relatively high value for stress-at-break of 3.7 MPa at a strain-at-break of 79 %. This result was achieved in an environment of pH 3.0 while in the case of pH 7.4 lower values of 2.6 MPa and 60 %, respectively, were obtained. The effect of increasing compressive properties occurred in correlation to higher pH values and polymer volume fractions.

REINFORCED WITH HYDROXYAPATITE NANOPARTICLES COLLAGEN HYDROGELS AS A BASIS FOR THE BONE TISSUE ENGINEERING

NATURAL BONE TISSUE COMPOSITION AND STRUCTURE ORGANIZATION

Bone grafts are the second most common transplantations tissue with expected market of about 2.5 billion US $ [79, 80]. Bone is composed of two major constituents: a strong protein matrix, rich of collagen nanofibers (collagen hydrogel is around 30 wt. % of the organic bone phase), and bone minerals (hydroxyapatite (HA, [Ca₃(PO₄)₂]·Ca(OH)₂) nanocrystals are about 60 wt. % of the inorganic phase of the bone tissue), deposited in this matrix to make it hard and nonflexible [81, 82]. Bone hierarchy consists of structural units with two different length scales. At the macrostructural level there are spongy (20 % of the total bone) and compact (80 % of the total bone) bones with a quite different density [83]. At the nanostructural level, the bone tissue consists of collagen nanofibers and bone HA nanocrystals. Therefore, the bone is considered as a nanocomposite, constituted of nanosize crystals of HA, grown in intimate contact with a protein matrix, rich of collagen nanofibers [84, 85].

Bone grafting is a surgical procedure that replaces missing bone in order to repair the bone fractures. The main requirements to the successful bone grafts are mechanical characteristics close to those of the natural bone, osteoconduction (guiding the repature growth of the natural bone), osteoinduction (encouraging undifferentiated cells to become osteoblasts) and osteogenesis (living bone cells in the graft material contribute to bone remodelling) abilities [86]. The requirements to the HA-consisting bone scaffolds are: i) to be three-dimensional and porous with interconnected pores to offer channels for migration of host cells and flow transport of nutrients and metabolic waste into the matrix which can
serve as substrate for cell attachment, growth, tissue formation; ii) to be biocompatible and biodegradable with a controllable degradation and resorption rates; iii) to have suitable surface chemistry for cell attachment, proliferation and differentiation; iv) mechanical properties that match those of the tissues at the site of implantation [87–89]. HA has excellent biocompatibility, bioactivity and osteoconductivity.

Therefore, it is applied as a scaffold material for bone tissue engineering. Although much efforts have been put into the development of porous HA scaffolds with mechanical characteristics suitable for bone regeneration, the inherent lack of strength of HA scaffolds is associated with their porosity. Pore sizes more than 300 μm are recommended for enhanced new bone formation [90, 91]. Therefore, the developed method for synthesis of porous HA scaffolds with unusually high comprehensive strength (up to 145 MPa for 56% porosity and 65 MPa for 56% porosity) [92] by freeze casting is a considerable achievement in this direction. Another solution to this problem is proposed by Lickorish et al. [93]. A bone graft substitute comprising of a porous collagenous scaffold (porosity of approximately 85%) was biomimetically coated with HA using a simulated body fluid solution. Glutaraldehyde vapor was used to cross-link and stabilize the collagenous scaffold. Strong Coll/HA nanocomposite scaffolds with super high porosity (98.9%) were also produced by two original methods [94]. According to the first one HA suspension was added to the collagen slurry (suspension method), and according to the second one, porous collagen scaffold was immersed in nano HA suspension (immersion method). The Coll/HA nanocomposites produced by these methods display excellent bioactivity and potential for graft subunits in orthopaedic regenerative medicine. It is interesting also that the electrically charged HA ceramics has a selective cell adhesion and show that polarized HA offers significant effects on bone cell growth and adhesion [95].

The bone extracellular matrix is rich of collagen, that contributes to mineral deposition, vascular ingrowth, and growth factor binding, providing a favorable environment to bone regeneration [90, 96, 97]. Triple helix structure is a peculiarity for collagen macromolecules, which consists of two identical chains and an additional chain with different slightly chemical composition. Atypical for proteins is the amonoacid collagen composition. High hydroxyprline (Hyp) content and Glycine-Proline-X (Gly-Pro-X) and Gly-X-Hyp (X is any amino acid, other than Gly, Pro and Hyp) are the most common motifs in the amino acid sequence of collagen macromolecule [96, 98]. In vivo, multiple collagen fibrils form collagen fibers with a diameter of 67 nm [99]. These fibers are the major component of the extracellular matrix and support the cell structure [100, 101]. Although 28 types of collagen are known, the bone organic phase is formed from type I collagen. Collagen is widely employed in bone substitutes [96] and some commercial products from collagen are on the market, such as Collapat II® (Biomet Inc), Collagraft® (Nuecoll Inc., Zimmer Inc.), Biostite® (Vebas S.r.l.) [102]. Collagen remains the most important biopolymer for the engineering of bone mimetic tissue because of its biocompatibility, biodegradability and bioactivity. Collagen-based composites with HA and some other noncollageneous matrix proteins (osteocalcin, osteonectin, thrombospondin, morphogenic proteins, sialoprotein, serum proteins, osteopontin; approximately 3–5% of the bone) are currently the major bone tissue biomimetics.
Predominantly the minerals (HA, carbonates, citrates, etc) are not directly bounded to the collagen, but they are bounded through the mentioned above noncollageneous proteins. This interaction is often called biomineralization, whose mechanism is still not fully understood. The non-collageneous proteins provide active sites for biominerlization. Wang et al. [103] show that the collagen matrix influences the structural characteristics on the atomic scale, and controls the size and the 3D distribution of HA at larger length scale.

Glycosaminoglycans (GAGs) (other components of the bone organic phase) are long unbranched polysaccharides consisting of a repeating disaccharide unit and amino sugar (N-acetylglucosamine or N-acetylgalactosamine) along with a uronic sugar (glucuronic acid or iduronic acid). Examples of GAGs include chondroitin sulfate, keratin sulfate, heparin, heparin sulfate, hyaluronic acid, etc. [104]. By covalent binding to protein they form proteoglycans. It is well known, that the structural proteins of ECM are augmented in their biochemical functions by glycosaminoglycans [90]. These strong anionic biopolymers absorb water, which provides compressive strength to the ECM, while the glycosaminoglycans also directly affect tissue organization via interaction with cell-surface receptors [105, 106]. Hyaluronic acid adsorbs enormous amounts of water at equilibrium and forms a non-fibrilar hydrogel. The latter have been used for various applications, including chondrocyte transplantation for cartilage repair [107, 108]. Cellular infiltration in the hyaluronic acid hydrogel can be improved by using morphogenic peptides (MPs)-sensitive cross-linkers [109].

The inclusion of HA nanoparticles into the biopolymer matrix (collagen, chitosan, hyaluronic acid, etc.) improves the mechanical properties and incorporates the topographic features that mimic the structure of bone [110]. The properties of such composed materials can be tailored by the addition of different organic and inorganic components: poly(vinyl alcohol), collagen hydrolisate, ionic species (citrates, florides, chlorides, carbonates), adhesive proteins, growth factors, etc. [111–116].

Porous scaffolds with good mechanical properties were prepared via liophilization of frozen hydrogels made from collagen, modified with chitosan nanofibers, hyaluronic acid, poly(lactic-co-glyconic acid) and HA nanoparticles [117]. The modified collagen composition was cross-linked with N-(3-dimethylamino propyl)-N′-ethylcarbodiimide (ECD), combined with N-hydroxysuccineimide (NHS) in water solution. Collagen scaffold modified with hyaluronic acid presents reduced deformation at break, while the presence of HA enhances the scaffold deformation under tensile loading. The tensile elastic modulus of chitosan nanofibre collagen scaffold is the lowest, but closest to the articular cartilage. However, the strength and the deformation the failure increases up to 200 %. The cross-linked collagen/chitosan network after premineralization with Ca\(^{2+}\) and phosphate salts in simulated body fluid (SBF) also demonstrates a significantly improved mechanical strength [118]. If the collagen/chitosan precipitation is performed in the presence of HA and the quick freezing suspension is lyophilized, the highly porous scaffold with good mechanical properties and healing potential is produced also produced [119].
Coll/HA scaffolds prepared by a phase separation technique and cross-linked by (EDC)/(NHS) (used also for covalently attachment of the chondroitin sulfate to the scaffold) have good cell biocompatibility and osteoinduction after the bone morphogenic protein incorporation into the scaffold by adsorption. Attachment of chondroitin sulfate improves the cell adhesion and differentiation [120].

Coll/glycosaminoglycan (GAG) scaffolds for bone tissue engineering were also prepared and tested [94, 121, 122]. It was shown that the collagen/GAG concentration ratio and conditions for the dehydrothermal cross-linking are the effective parameters for a control of the mechanical stiffness and tensile properties. Such Coll/GAG scaffolds with an optimal composition, pore structure and distribution (produced by controlled freeze-drying processes) show immense possibilities for bone repair [80]. After the introducing ceramic components (HA, \(\beta\)-tricalcium phosphate etc) in these collagen-based scaffolds their mechanical properties, porosity and vascularization ability are considerably improved. The investigation of the influence of the pore size ranging from 85 \(\mu\)m to 325 \(\mu\)m [123] on the osteoblast adhesion and proliferation shows that the pore size of 325 \(\mu\)m is better for vascularization of this scaffold.

HA/Coll nanocomposite was also prepared using the preliminary phosphorilated collagen as a nucleation site for the subsequent bone-like HA mineralization [124] in the simulated body fluid. The obtained nanocomposite exhibits similar composition and crystal morphology as in natural bone. It was shown that biomaterial composed of sintered and powdered HA and type I collagen, both of bovine origin, have osteoconductive and osteoinductive activity [125]. NanoHA/Coll composite with some features of natural bone in both composition and microstructure was also developed [126]. The composite consists of up to 50 wt.% carbonate substituted nanoHA precipitate, uniformly distributed in a type I collagen matrix without preferential orientation. Its ability for bone repairing was confirmed. An original biomimetic self-assembly method was developed [127] to create nanocarbonated HA/collagen (nanoCHA/Coll) composites by incorporating of various collagen and carbonate concentrations using solutions such as \(\text{CaCl}_2\), \(\text{H}_3\text{PO}_4\) and \(\text{Na}_2\text{CO}_3\). By manipulating the concentrations of collagen and carbonate, various morphologies of the nanoCHA/Coll can be obtained. Shibata et al. [128] change HA with \(\beta\)-tricalcium phosphate, which biodegrdability provides better osteoconductivity than HA. Bonding strength between collagen amino groups and \(-\text{P}−\text{O}–\text{P}−\text{ in this case increases as compared to those in the nanoHA/Coll composite. Dawson et al. [129] develop specific collagen scaffolds to support the osteogenic and chondrogenic differentiation of human bone marrow stromal cells based on nanoCHA/Coll composites.}

Proteins that contain the tripeptide Arg-Gly-Asp (RGD) attachment site (half of the over 20 known integrins, other cell surface proteins, fibronectine) constitute a major recognition system for cell adhesion and cell-cell interaction. Such peptides promote cell adhesion when insolubilized onto a surface, and inhibit it when presented to cells in solution [105, 130–134]. Therefore to increase the bioactivity of Coll/HA biomaterials they are coated with ECM macromolecules such as fibronectin, elastin, lamini, containing RGD tripeptide, as well as YIGSR, GROGER, other signal peptids [135].
The application of scaffolds as delivery systems for therapeutic genes is a new, promising approach of the gene therapy and can thus be a valuable tool to avoid the limitations of the local delivery of growth factors [136].

**Vascularization of artificial bone tissue materials**

The main direction of the bone tissue engineering in the last years is shifted from the osteoblast, osteoconductive and osteoinductive properties of the bone tissue grafts to their vascularization ability [137] by the addition of angiogenetic growth factors, the seeding of mature and progenitor endothelial cells and the creation of microcapillary-like structures into scaffolds [138–141]. Therefore, the last generation of the tissue engineering methods combines the reinforced with Coll/HA hydrogels with five types bone matrix cells (osteoblasts, osteocytes, osteoclasts, osteoprogenitor and bone-lining cells), different growth factors, bone morphogenic proteins, etc [142, 143]. The results obtained demonstrate that substrate stiffness can regulate the both the behavior of mature cells and the differentiation pathway of stem cells [144–147]. For example, when mesenhimal stem cells (MSCs) are grown on elastic gels that mimic elasticity of muscle, differentiation down a myogenic (muscle forming) lineage is observed, whereas when MSCs are grown on rigid gels that mimic precalcified bone, the cells differentiate down an osteogenic pathway. Therefore, increasing research efforts are directed to the utilization of this cell mechanosensitive capacity.

To this aim a method for the incorporation of microchannels, able to accommodate blood vessels and to permit the flow of nutrient-rich media through collagen-based scaffolds is developed [148]. Another approach to this aim is drying at CO₂ critical point [149, 150]. Critical point drying did not induce elemental, crystallographic or molecular changes of HA. An unique pore structure with unidirectorially interconnected pores was fabricated by unidirectional growth of ice crystals using a cooling stage and a subsequent freezy-drying process. The porous composite showed an elastic property and anisotropic compressive The Coll/HA weight ratio influence on the flexural modulus (with maximal value 2.46 MPa) and strength (with maximal value 0.65 Mpa) was investigated by this authors also [151].

Original biodegradable bone substitute materials were proposed by Fukui et al. [152] on the basis of nanoHA and special type I biodegradable honeycomb collagen sponge (HCS). Three weeks after their implantation into the bone defects the bone formation is the largest compared to HGS only and HCS/calcinated HA as comparative materials. It means that nanoHA/HCS composite can be orposed as an effective biodegradable bone substitutive material. Similar biocompatible high porous collagen based scaffold with incorporated osteoinductive HA particles was also proposed [153]. It exhibits improved mechanical properties, healing potential and bond defect repair ability. A multilayer nano-composite scaffold was also prepared [154], which after the colonization with chondroctes demonstrates high cartilage regenerative potential. Highly oriented Coll/HA nanocomposite materials were prepared also through the self-assembling method using collagen gel (3.2 % aqueous collagen solution) and successsively the addition of Ca(OH)₂ suspension.
and NaH$_2$PO$_4$ solution to it at 37 °C and pH 9. A feasible approach to prepare a bone tissue with fibrovascular network structure was developed by Cheng et al. [155], using type I Coll/HA beads immersed in platelet-rich plasma, the natural source of the growth factor.

To overcome the limitation for the re-loading of scaffolds with bioagents after implantation, the magnetic biomimetic scaffolds were developed [156]. The latter are able, via magnetic driving, to attract and take up in vivo growth factors, stem cells and other bioagents bound to the magnetic particles. To this aim, the standard HA/Coll scaffolds are dip coated with ferro-fluids, containing iron oxide nanoparticles. By this way these nanoparticles are integrated into the scaffold structure, providing the latter the suitable magnetization. It was proved that the magnetic scaffold do not release iron oxide particles under a constant flow of simulated body fluid and support adhesion and proliferation of the human bone marrow stem cells in vivo. The strong magnetic field (10T) imposed perpendicularly to the rotation axis of the mixed collagen calcium phosphate solution was used for the production of the unidirectional oriented Coll/HA nanocomposite [157, 158]. The electrical field was also used to this aim [159].

**Bone tissue on the basis of the electrospun produced bio- and synthetic nanofibers**

Synthetic and natural polymer electro-spun nanofibers are explored as scaffolds, similar to nature ECM [160, 161]. The most important processing parameters of this method are spinning voltage and polymer solution concentration. They determine the morphology and size of the fibers produced. Suitable mechanical properties of the collagen matrix can be tailored by controlling the fiber orientation [162]. Electrospun type I collagen, solved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP) was used for preparation of the nanofibrous ECM for tissue engineering [163]. Electrospun polycaprolactone/nanoHA/Coll was used for preparation of the biocomposite nanofibrous scaffold with good mechanical properties and adhesion to human fetal osteoblasts [164, 165]. Electrospun Coll/nanoHA composite materials with different Coll/HA weight ratio were also investigated [166]. The values from 0.2 GPa to 20 GPa for Young’s modulus and from 25 MPa to 500 MPa for hardness were registered depending on the composition and microarchitecture of the composites. Electrospun Coll/HA nanofibrous scaffolds were prepared and used in the presence of the osteoblast cells [167, 168]. The matrix was chemically cross-linked by glutaraldehyde vapor and then treated with 0.1 M aqueous glycine solution to block unreacted aldehyde groups. The produced collagen nanofibers are very effective as wound-healing accelerators.

It is important to note that many properties of collagen are lost (99 % of its triple helical structure) during its electrospinning from HFP and other fluoroalcohols [169]. To overcome this limitation the water/alcohol/salt complex solvents was proposed [164].

The methods for electro-spinning of the uni- and co-axial aligned collagen nanofibers were also developed [170–174]. The collector consisting of two pieces of electrically conductive substrates, separated by gap with different width is used for this purpose. The production of the core-shell nanofibers through electro-spinnig was also developed [173].
CONCLUSION

Hydrogels with enhanced and controllable mechanical, structural properties and porosity find an increasing medicopharmaceutical application i.e. as tissue materials and target drug delivery systems. Therefore, the critical and analytical review of different approaches for the preparation of mechanically strong hydrogels with designed mechanical characteristics, porosity and biocompatibility is of value. The main three groups of hydrogels each with different kinds of reinforcing factors were distinguished and their equilibrium water contents and mechanical characteristics were collected and compared. In all cases, the highest compression stress-at-break of 17.2 MPa was obtained for PAMPS/PAAm double network hydrogels, while the maximum strain-at-break was found for polyglycole/nano-hydroxyapatite at 1970 % in tensile test measurements.

From the discussion above it becomes clear that collagen hydrogels remain the most important starting material for engineering of bone mimetic tissue because of its biocompatibility, biodegradability and bioactivity and the possibility to control its mechanical characteristics adding hydroxyapatite and other biocompatible minerals. Collagen based nanocomposites with hydroxyapatite, some other noncollageneous matrix proteins and glycodaminoglucans are currently the major bone tissue biomimetics. The poor angiogenesis of the engineered bone biomimetics is the general challenge, and the vascularization strategies (mentioned partly) for the improvement of this tissue characteristic is under the most extensive research. The addition of the active molecules in collagen based bone biomimetics and, containing vascular cells is a way to solve this "problem.

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REFERENCES

Резюме. Описани са методи за получаване на три основни вида хидрогелове (двойни мрежи, нанокомпозити, съполимерни или полимерни смеси) с усилени механични отнасяния. Медико-фармацевтичните приложения на хидрогелове на основата на колagen, смеси на колаген с неколагенови протеини и смеси на колаген с глюкоаминоглюкани, усилени с нано-хидроксиапатит и/или бета-трикалциевифосфатни наночастици са представени като пример за материали, използвани за най-често срещаните тъкани присадки (на втора позиция), каквито са костните.

Ключови думи. Хидрогелове, Механично напрежение, Разтягане при скъсане, Модули на натиск и на разтягане, Колаген, Неколагенови протеини, Глюкоаминоглюкани, Костна тъкан, Костни присадки, Извънклетъчна матрица, Остеорегенерация, Остеоиндуциране, Остеоинтегриране, Остеогенни клетки, Васкулизация на костната тъкан.

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