Methods for obtaining structural information on GelMA hydrogels

Bente van Braam

S3481220

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Supervisor: Prashant K. Sharma

Abstract

Hydrogels composed of crosslinked networks of polymers, such as polyethylene glycol, GelMA and alginate, are often used for 3D cell culture or as cell-laden biomaterial implants to promote tissue regeneration. hydrogels are porous gel structures which have high water content. Researches showed that both the physical and biochemical properties of the hydrogel play a major role in regulating cell behaviours. To better understand the impact of hydrogel structure properties on cell behaviours,, different methods are used to image these structures. Currently scanning electron microscopy is used most, because of the details that can be seen. One of the problems which are encountered is the drying of the sample in the sample preparation and nonconductive samples need to be sprayed with Au and Pt therefore this literature survey is looking into all the methods or techniques that can be used best to obtain structural information on hydrogels.

Summary

Gelatine Methacryloyl hydrogels, short GelMA hydrogels are a gel like material, which is crosslinked to form a network, which can entrap water. The pores of GelMA hydrogels are 5 µm to 150µm big and are distributed throughout the hydrogel. To map these pores, different techniques are used. The methods surveyed in this survey are divided into three topics ; microscopy, scattering methods and analytical methods. In the microscopy part, the Scanning Electron Microscope, in short SEM, is being discussed and compared to the Confocal Laser Scanning Microscope, in short CLSM. The biggest disadvantage of SEM is that the hydrogel samples need to be dehydrated, therefore the confocal laser scanning microscope is a better option. But the CLSM has the disadvantage of the invasive staining again. Also the atomic force microscope is being looking into, but this technique is not suited for the “big” hydrogel pores.

Diffusing Colloidal Probe Microscopy is a technique that uses colloidal beads and the position of those beads to obtain structural information on hydrogels, but this technique is not yet used to determine the pore sizes. It’s promising for experimenting with it.

The scattering methods are very promising, due to the fact that the samples are not dried, but maybe the Small-Angle X-ray Scattering method is already too detailed to detect pore sizes, but the method can be used to obtain other structural information.

The analytical methods are divided into two parts, two methods, Nuclear Magnetic Resonance and Fourier Transform Infrared Spectroscopy are used for obtaining information on a molecular level. Therefore not suitable for mapping pore sizes. But the combination of CLSM and an algorithm, in the 3D bubble method might be a very interesting technique, but more experiments need to be done, to determine if this method can be used.

Introduction

Hydrogels are the most common manmade porous materials used in studies, which research aqueous lubrication[1]. Hydrogels consist of a crosslinked network of polymer chains, which are swelled with water. Those synthesized polymers can be made of a biomaterial and therefore inert in the human body. Hydrogels are synthesized such that different properties can be altered. Examples of properties include elasticity, strength, but also water content and pore size.

Hydrogels are widely researched, because of promising appliances in the biomedical field. Gelatine Methacryloyl, short GelMA, is a biomaterial obtained from the hydrolysis of collagen, the most abundant protein in the human body[2]. Raw gelatine is relatively cheap and easy to obtain, it can only form a hydrogel under specific conditions. The product made; GelMA, is useful in different biomedical applications. Under which artificial extracellular matrix, short ECM. ECM is the non-cellular part of tissues, which functions as support for cells; it's secreted by specific connective tissue cells[3]. ECM contains a large amount of water in its porous structure, the structure looks like a hydrogel and therefore ECM can be seen as a natural hydrogel.

GelMA hydrogels are synthesized such that the different properties can be altered. The structure of the GelMA hydrogels are synthesized such that the different properties can be altered. The structure of the porosity affects the diffusion of biological molecules, such as growth factors for wound healing in the ECM. To map those structures, different methods are needed.

Two wide-ranging ways to prepare hydrogels for mapping structural properties are; hydrated or dried (mainly freeze-dried). Literature suggests that there is a big difference between freeze or air-dried hydrogels relative to hydrated hydrogels. The water trapped in the hydrogel network is evaporated in dried hydrogels, therefore leaving void spaces between the cross-linked polymers. This might cause the problem of inconsistent structural imaging. Therefore, the research objective of this literature survey is to determine methods or techniques available for obtaining structural information of hydrogels.

In this survey the focus is on the structural properties of hydrogels; Structural properties are properties that express information about the role of the elements in the overall structure of the system[4]. To specify even further, this survey is on the GelMA hydrogels.

All methods have different advantages and disadvantages, it can have limitations in the resolution and image quality, but some methods also have limitations in sample preparation. Challenging limitations cause no method to be perfect. Microscopy provides detailed images on the structure of the hydrogels, those images are real-time images. Neutron and X-ray scattering are techniques which uses the scattering curves of the neutrons or photons and analytical methods show us for example the resonance of the hydrogen elements, which can be used to define the molecular composition.

GelMA

Gelatin Methacryloyl, short GelMA, hydrogels are made from porcine-derived gelatin, crosslinked with methacrylic anhydride. These hydrogels have promising properties and are suitable for use in biological applications. GelMA hydrogels behave similarly to natural ECM, due to their presence of matrix metalloproteinase responsive peptide motifs and the cell attachment[5]. Cells can disperse through the cell and they can proliferate.

The presence of gelatin makes the hydrogels biocompatible and on top of that, the hydrogels are easily modified. By altering the amount of methacrylic anhydride compared to the gelatin, the GelMA hydrogel properties can be adjusted.

Van Den Bulcke et al.[5] developed the method of synthesizing GelMA hydrogels. The gelatin is added to a phosphate-buffered saline solution at 50 degrees Celsius. The methacrylic anhydride monomers to this solution, where these monomers reacted with the hydroxyl lysine groups of the gelatine molecule. Hitomi Shirahama et al.[2] stated that the synthesis of the GelMA is still not optimal, there is room for improvement of controllability and efficiency.

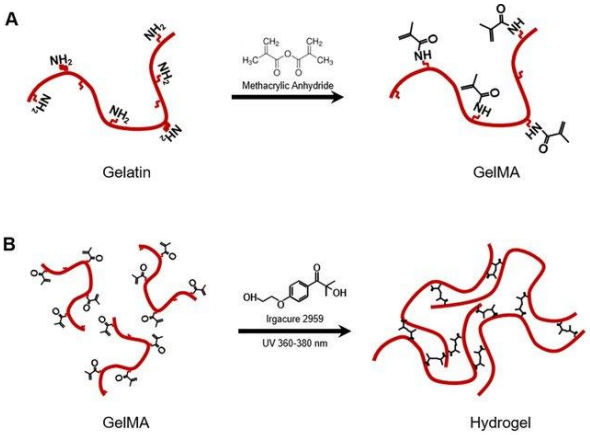
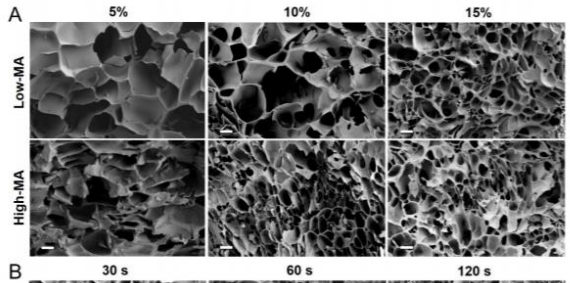


Figure 1 – a schematic representation of the synthesis of GelMA hydrogels. Gelatin molecules react with Mehacrylic Anhydride to form polymers. The GelMA polymers are cured under a UV light, to form the hydrogel. [6]

In theory the methacrylic anhydride monomer can only react with one lysine group, but several studies suggested that by adding an enormous amount of methacrylic anhydride, the degree of substitution will be at a higher level than equal amounts of the two molecules. The degree of substitution has an effect on the structure of the GelMA hydrogels. A high degree of substitution is found in relation with the stiffness of the hydrogel[7]. Therefore the structural properties are also adjusted.

Amir K.Miri et al.[3] did research on the permeability mapping of gelatin methacryloyl hydrogels, therefore they imaged the pore sizes of the GelMA hydrogels. The samples were prepared with different amounts of methacrylic anhydride monomers, so the network is altered. They looked into the pore sizes to determine structural information on the GelMA hydrogel. Their results are presented in figure 1. Taken into consideration the standard deviation; the pore sizes range from 5µm to 144µm.



C

	5%	10%	15%
Low-MA	96 ± 48 µm	61 ± 29 µm	39 ± 21 µm
Medium-MA	62 ± 31 µm	43 ± 19 µm	30 ± 18 µm
High-MA	45 ± 27 µm	36 ± 17 µm	19 ± 14 µm

Figure 2 - SEM of Gelma microstructure with different concentrations of MA. Pore sizes from ~5 µm to 150 µm are seen.

The techniques reviewed in this literature survey are looking into the structural information of GelMA. The pores of the GelMA are 5-150 µm big, therefore this size needs to be taken in consideration when discussing the techniques.

Amir K.Miri et al.[3] sampled three different hydrogels, with different amounts of methacrylic anhydride, to alter the diffusion rate of certain drugs. Their results showed indeed different releasing rates of the drug, therefore one of the biomedical applications of GelMA hydrogels would be drug release. To control this drug release, the pore sizes must be mapped correctly.

Gewijzigde veldcode

Methods and Materials

Smartcat

Keyword	Amount of articles found	How many selected
Hydrogel	25.915	0
GelMa hydrogels	365	4
Structural properties hydrogels	2623	3
Drying Affect Gel Networks	1311	2
Techniques measuring hydrogels	685	1
Methods measuring uniformity hydrogels	216	1
Mesh size hydrogels	297	1
H-nmr hydrogels	2973	1
saxs hydrogels	178	2
Autofluoresent hydrogels	1	1
NMR hydrogels	1430	1
Differential scanning calorimetry hydrogel	1719	2

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Inclusion and exclusion criteria Smartcat

- All libraries
- Peer reviewed
- Articles
- Last 5 years, but if no useful articles were found, I expanded it to the last 10 years

Google scholar

Keyword	Amount of articles found	Which selected
Hydrogel	87.600	0
Structural properties hydrogels	16.400	1
Techniques measuring hydrogels	16.200	1
Confocal Laser Scanning Microscopy hydrogels	17.400	1
SAXS hydrogels	5.740	1
3D bubble analysis algorithm	17.600	1

Inclusion and exclusion criteria google scholar

- From 2017 and up

Pubmed

Keyword	Amount of articles found	Which selected
Hydrogel	22,488	0
Atomic Force Microscopy	11203	1

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Diffusing colloidal probe microscopy	864	1

Inclusion and exclusion criteria Pubmed

- From 2017 and up
- Full text available
- All article types

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Results

As explained in the introduction, hydrogels are ideally characterized in the hydrated form. Different techniques have been experimented with the methods to conclude various techniques to be better relative to others.

The question of the effects of dehydrating hydrogels arises in more and more articles, because various microscopic techniques require drying the samples. Dave J. Adams[8] reviewed the effects of drying hydrogels. As also stated in the reviews, artifacts in the hydrogels are suggested, but it's rarely acknowledged in the literature. They reference a recent review that provided some examples of drying artifacts, in this article they wrote about the dangers of drying soft materials like hydrogel. Linda E Franken et al.[9] highly discourages dehydrating soft materials and came upon the conclusion that the results of hydrated and dehydrated samples are often overlooked.

Dave J. Adams[8] states that artefacts in the structure of gel materials are possible, therefore they suggest that researchers should provide their research with the question if the dried hydrogels indeed show the realistic structural properties, because sometimes the assumption is made that the data for dried gels represents the data of the wet gels.

Therefore in this survey, the dehydration of the hydrogels is taken as a big disadvantage of a method or technique.

Microscopy

Microscopic techniques are unique tools to obtain structural information of materials. They produce real-time images of the material, but some techniques require the drying of the sample. However, the disadvantage of microscopic techniques is the limited penetration depth. Early researchers, who wanted to have a detailed look on biological materials, made thin slices of their samples. This was limited to dead tissues, because the samples needed to be fixed.

The shift to more modern microscopic techniques has shown various great results in the 3D mapping of living material. But still those techniques have their limitations, mostly in sample fixation.

Scanning Electron Microscopy

When producing an image with a Scanning Electron Microscope, short SEM, the samples are scanned with an high-energy beam of electrons. Those electrons cause characteristic X-rays, secondary electrons and backscattered electrons, which are detected to create an image[10]. The different detected electrons are dependent on characteristics of the sample.

To prepare the samples for this method of microscopy, they need to be dried. There are different ways to dry samples, of which freeze drying and critical point drying are most commonly used[11]. The size of the details which can be imaged with a convention SEM microscope range from approximately 1cm to 5 µm[12]. Which is detailed enough to show the pores of GelMA hydrogels and other structures visible in the hydrogels.

Laser Scanning Confocal Microscopy

Laser Scanning Confocal Microscopy, in short LSCM, uses fluorescence properties of materials. The samples are labelled and the LSCM excites this dye with a laser, the labelled sample parts emit red-shifted light, which is detected to make an image. Stefan M. Paterson et al.[11] labelled the sample in an aqueous environment, so the hydrogels were kept hydrated. Dehydration can damage the hydrogels, therefore this method has an advantage relative to other methods. In this experiment the dye did not affect the quality of the images obtained from the LSCM, but sometimes the labels have a negative effect on the material. In the experiment they concluded that the images obtained are images that represent the actual structure of the PHEMA hydrogels.

Discussion LSCM and SEM

Stefan M. Paterson et al.[11] experimented with poly-2-hydroxyethyl methacrylate (PHEMA). They obtained images with Laser Scanning Confocal Microscopy and Scanning Electron Microscopy. The article discusses both methods. PHEMA has pore sizes ranging from 50 µm to 200 µm[13] and therefore relatable to GelMA hydrogels.

In the experiments performed by Stefan M. Paterson et al.[11], the SEM method showed more details looking at the images obtained. A significant problem with SEM sample preparation is that they must be dried in the sample preparation. Of course the removal of the hydrogels is done, such that the morphological structure remains as realistic as possible. As stated by Stefan M. Paterson et al.[11] the dehydration of the hydrogels has a huge impact on the structure, this is substantiated by[14]. The experiment conducted with both methods showed different morphological features, with different methods of drying.

Using LSCM as a technique to map the structure of the PHEMA hydrogels, the sample preparation did not involve any drying of the hydrogel. SEM sample preparation dehydrates the hydrogels and therefore creates artifacts in the structural properties of PHEMA. This is mainly because drying or freeze drying causes crystallization of water and this has a different effect on the network in comparison to the non-crystallized water in the network. In this research, the hydrogels are limited to only PHEMA based hydrogels, so the artifacts shown in SEM sample preparation might not be the case in other hydrogel structures. Still, the hydrogels are dried, so it might cause similar issues.

The conclusion made by Stefan M. Paterson et al.[11] is Laser Scanning Confocal Microscopy is a better option than SEM in mapping the structural properties of hydrogel.

Fluorescent microscopy

A disadvantage of fluorescent microscopy, like LSCM, is the labelling of the samples. Jun Chen et al.[15] did research on certain hydrogels, which already contain fluorescent properties. The hydrogels therefore do not need to be labelled. They reported a bovine serum albumin hydrogel, which has auto fluorescent properties, so by looking at it under a normal fluorescent microscope, the structural properties can become accessible

This technique is probably not suitable for GelMA hydrogels, because there is no report on autofluorescence for this hydrogel. But if bovine serum albumin hydrogels are used, just normal fluorescent microscopy can be used to determine structural properties, without the invasive staining of the gels.

Atomic Force Microscope

An option to map the morphology of certain structures, like a hydrogel is by using an atomic force microscope, short AFM. An AFM has a scanner, a probe, a photodetector and a computer. The probe slides over the sample and the forces between the tips and the sample can bend the cantilever[16]. This bending is detected by a photodetector, so an image is formed.

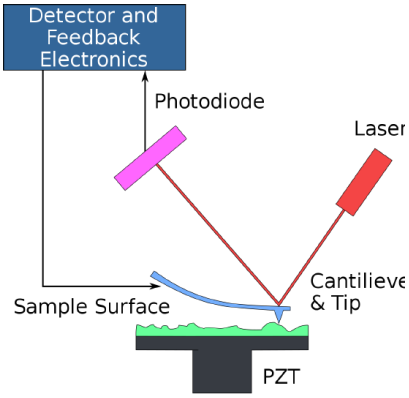

Figure 3 - a visual representation of the atomic force microscope[16]

The applications of the AFM are versatile, it can analyse various kinds of samples. Most of the time AFM is used to map the morphology of the samples and so also the morphology of hydrogels. The sample sizes are different with different machines, but most of the time 8mm by 8 mm, with a height of 1 mm. The tip of the AFM is very small and the characteristics of a sample can be mapped when the sizes range from 1 nm to 8µm[17], therefore the pore sizes are most of the time too big for GelMA hydrogels. Probably some other structural information can be obtained, for example how the polymers are attached to each other.

Diffusing colloidal probe microscopy (DCPM)

Soheila Shabaniverki and Jaime J. Juárez[18] studied the viscoelastic properties of gelatine hydrogels using both optical video microscopy combined with diffusing colloidal probe microscopy, short DCPM. DCPM is a method that tracks a colloidal bead using video microscopy, the position is determined using the potential energy interaction between the bead and the surrounding surfaces. These measurements are done in an aqueous environment, which would mean this technique can be used for hydrogels. In the experiment they embedded a gelatine hydrogel with colloidal beads, and when the probe flows above the beads, the elasticity can be measured. Maybe when altering the analysis this method can be used to determine the structure of the surface of the hydrogels.

Gregg A. Duncan et al.[19] developed another alternative algorithm to identify the position of both cells and the colloids. In their experiment, they used the analytical tools of CDPM to explore the dynamic and equilibrium surface interactions, therefore it can be promising to determine the surface interactions of hydrogels.

Scattering methods

Small-angle scattering methods are becoming increasingly important to obtain information about the structural properties of biomaterials[20]. The scattering method use the angle of the small deflection away from the primary beam, caused by the structure of the sample. This angle of deflection of the radiation is obtained and used for information about the size and the shapes of the material. Different setups of scattering methods make that there is small angle scattering X-ray, but also Ultra-small-angle scattering x-rays and wide-angle scattering x-ray are methods using the scattering of radiation to obtain information of the samples.

In the scattering techniques, high energy photons and neutrons are both considered to be waves of radiation. Between energy and wavelength there is a simple relationship. These formulas are different for x-rays (high energy photons) and neutrons.

The basic principles, only elastic scattering is taken into account, which means that no energy is transferred during the scattering process. This elastic scattering is most useful in studies of structural properties.
The angles of the scattered photons are specific to different atoms, this can be used to determine structures. The scatterers have a position in space, which can be transformed into real coordinates, this is done with Fourier transformation.

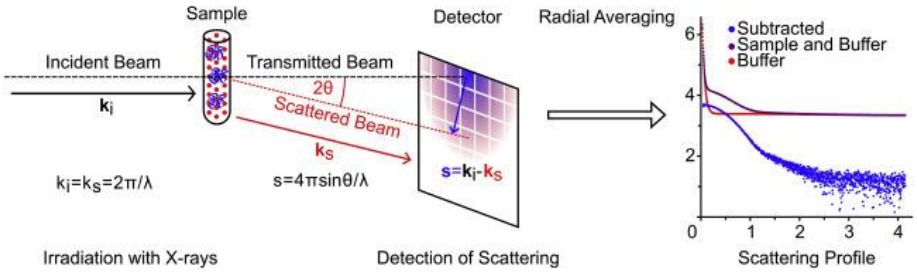

Figure 4 - a visual representation of scattering techniques[20]

Small-angle x-ray scattering

Tobias W.Gräwert and Dmitri I.Svergun[21] reviewed small-angle X-ray scattering, short SAXS, to examine shapes and sizes of macromolecules. SAXS is a quick method that can characterize entire protein complexes in high resolution[21]. If the complete hydrogel structure can be mapped, the structural properties like the pore sizes can be determined in very much detail. Unfortunately the review stated that the details which can be seen in sample range from 10 to 1000 nm, which might be too detailed to detect the pore sizes of GelMA, But the method is very promising to obtain structural information of the cross-linked network, but more in detail than just pore sizes.

One of the bigger benefits is the fact that the hydrogels can be measured in their natural state. The hydrogels can be prepared in the capillaries made to fit in the SAXS machine. Everything in the solution and the capillary itself will scatter and the data will be collected. So it's better to understand the chemical properties of the hydrogels first, with usage of for example nuclear magnetic resonance.

SAXS compared to SEM

Tsukasa Miyazaki and Mikihito Takenaka[22] researched the promising SAXS technique to obtain information on the mesh sizes of hydrogels. But they also compared the results of pore size of the scanning electron microscopy and the small-angle x-ray scattering. The polymer samples experimented with are so-called track-etched membranes. This is polyethylene terephthalate (PET), of which the pore size is controlled from 10nm to 100 nm with the etching time. The GelMA have a pore size 5 µm to 150 µm, therefore the results of this research do not have comparable results as GelMA in case of the structural imaging of the hydrogels.

Gewijzigde veldcode

Tsukasa

Table 1
Sample preparation conditions and pore size and pore size distribution measured by various evaluation techniques.

Sample	Etching time (min)	Pore density (/cm^2)	Volume fraction of the pores (%)	Pore diameter (nm)[a]	Pore diameter (nm)[b]	Pore diameter (nm)[c]	σ (nm)[d]
A	15	3×10^8	1.91×10^{-2}	8 (1)	NA	9.0 (0.5)	2.0 (0.5)
B	21	3×10^8	8.05×10^{-2}	20 (2)	NA	18.5 (0.5)	4.0 (0.5)
C	30	3×10^8	1.59×10^{-1}	25 (2)	NA	26.0 (1.0)	5.0 (1.0)
D	35	7×10^6	4.01×10^{-3}	30 (2)	NA	27.0 (1.0)	4.0 (1.0)
E	40	7×10^6	5.63×10^{-3}	40 (3)	32 (6)	32.0 (1.0)	4.0 (1.0)
F	45	7×10^6	6.35×10^{-3}	45 (2)	35 (5)	34.0 (1.0)	7.0 (1.0)
G	50	7×10^6	1.72×10^{-2}	60 (4)	52 (5)	56.0 (1.0)	7.0 (1.0)
H	120	7×10^6	0.71	270 (15)	320 (30)	360.0 (5.0)	18.0 (3.0)
I	200	7×10^6	2.18	420 (27)	560 (32)	630.0 (10.0)	25.0 (5.0)

[a] Gas permeability measurements; the average values of four measurements and the standard deviations in the parentheses.
[b] SEM observations, the averaged pore diameter of five pores and the standard deviations in the parentheses.
[c] SAXS measurements, pore diameters and the standard deviations in the parentheses.
[d] pore size distributions and the standard deviations in the parentheses.

Table 1 - a summary of all the samples and their pore diameters[22]

Gewijzigde veldcode

Table 1. summarizes all the sample preparation, the SEM observations and the SAXS measurements. They concluded that for the samples A to D it was impossible to measure the size of the pores when looking at the scanning electron microscopy samples. The inconclusive results are due to the low resolution images. The SEM observations correlated with the results obtained from SAXS and those were also fairly consistent with the gas permeability measurements.

Their conclusion of the comparison between SEM and SAXS measurements is that the SEM is not detailed enough to determine all the pore sizes and SAXS should be used to determine the structure of the pores and the distribution of the pores. This might be the other way around for GelMA hydrogels, because the pores are much bigger than the pores of PET.

Conclusion SAXS

In conclusion; SAXS is a promising technique to determine structural properties of GelMA hydrogels. The determination of the pore sizes might be too big, but the structure of the pores and the characteristics of the network should be mapped out very well by SAXS.

Wide-angle scattering

Some SAXS devices have the opportunity to also offer wide-angle X-ray scattering, short WAXS. Both techniques use the same basic principles, but the WAXS scattering can be used with samples with a shorter range order. Thus, the main difference between WAXS and SAXS is the length scale, the WAXS technique can even illustrate at the subnanometer scale[23] .SAXS is used for bigger structures. Therefore both techniques are often used simultaneously, to make an image complete.

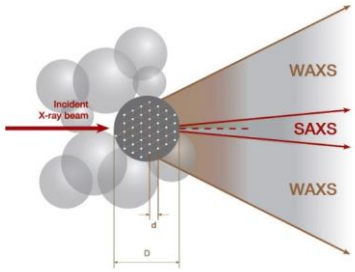

Figure 5 – a comparison of the angles of deflection detected by a SAXS and a WAXS instrument[24]

Ultra-small-angle x-ray scattering

Fan Zhang and Jan Ilavsky[25] did extensive research on Ultra-small-angle X-ray scattering, short USAXS. USAXS is capable of obtaining information about structure in the size range of 1 to 1000nm. Therefore promising in polymer research.

As stated in the introduction, this literature survey is specified into GelMA hydrogels are researched. Those hydrogels have a mesh size of ~5 µm to 150 µm, which is way bigger than the size range USAXS is capable of. Therefore this technique is probably excluded from using to obtain structural information on hydrogels.

Small-angle neutron scattering

Small-angle neutron scattering uses the same basic principles as x-ray scattering, neutrons are also considered radiation waves. But in contrast to X-rays, the neutrons will interact with the spin of the material and the nuclear potential. Neutrons are made in a reactor source, by a nuclear fission chain reaction or on proton accelerators on a heavy metal target. This needs to become a monochromatic beam.

The samples which can be measured using SANS can be bigger than the samples which can be measured by SAXS[20]. Therefore larger structures can be measured, hydrogels are big structures, so maybe SANS is a better option for determining the structure of GelMA, unfortunately this has not been done a lot to conclude if it will work.

Analytical methods

The above stated methods and techniques are able to make images from the hydrogels and when looking at those images, different structural information can be seen. Another approach are the analytical methods, most of the time there is a combination in an image and an algorithm to obtain this information. These combination might be very effective in determining pore sizes or for example the distribution of the pores.

3D bubble analysis algorithm

As stated in several articles, a physical parameter for determining effects of materials is the measurement of pore-sizes. Tony Fischer et al.[26] experimented with collagen matrices, they developed a two-step 3D pore-size analysis. Which might be a useful method for determining pore-sizes in hydrogels as well.

The experiments are conducted on 3D collagen matrices, they were stained and kept hydrated. Which would indicate a benefit in using it on hydrogels. Images were taken with a confocal laser scanning microscope. To confirm that the images were a correct representation of the collagen matrices, they are analysed with an atomic force microscope. Using the Bio-Formats library, the pore sizes were calculated. Different alteration by calculations are done, to obtain the actual pore-sizes.

The findings in this article states that the analysis is very well suited to describe the pore-sizes. They even detected the very small pores and also the pores that overlapped. They suggest that the method can be used on other matrices, such as gelatin gels. Only the algorithm needs to be adjusted to the material. Therefore this method is very promising to detect the pores of the GelMA hydrogels.

However, Fransisco Garcia Martinez (phd-student Medical Biology) tried this method on GelMA, but unfortunately it didn't work out. There are no confirmed reasons, but there is a possibility that the GelMa is stained too long to the staining substance. The pore sizes might also be too small or the visualization of the confocal microscope.

FTIR spectroscopy and solid state NMR spectroscopy

Helga Garcia et al.[27] research dextrin hydrogels by Fourier Transfer infrared spectroscopy. The work is about obtaining information on the structural modification taking place in the dextrin hydrogels. The characterization of hydrogels at a molecular level is done by the Fourier Transform infrared spectroscopy or with nuclear magnetic resonance spectroscopy.

This study two samples of dextrin hydrogels were made, one unmodified dextrine sample and samples with different degrees of the reactive vinyl acrylate groups. As stated in the

introduction, hydrogels contain a lot of water, in this study, no samples were dried before the analysis.

Both NMR and FTIR spectroscopy methods map the molecular structure of the hydrogels. This can tell us structural information, but not really on the pore sizes. To have an idea on how the hydrogel is formed on a molecular level, is could be necessary to understand the scattering methods.

Differential scanning calorimetry

Differential Scanning Calorimetry, short DSC, is a thermoanalytical technique that measures the difference in the amount of heat required to raise the temperature of a sample and a reference as a function of temperature. Throughout the experiment, the sample and reference are kept at nearly the same temperature. The temperature program for a DSC study is usually set up so that the sample holder temperature rises linearly as time passes.

Vladimir M. Gun'ko et al.[28] used this DSC method to determine the pore size distribution and they used CLSM to determine the pore sizes. This technique might not be very suitable for mapping the pore sizes, but it can obtain other structural characteristics, of which pore size distribution is one.

Gewijzigde veldcode

Discussion/conclusion

This literature survey listed several methods to obtain structural information of hydrogels, in particular GelMA hydrogels. Some methods are better than others, because of hydrogel preparation, but sometimes the old and conventional methods are still the ones used because of the best resolution.

	~~Sample sizes~~Details which can be seen in the samples	Sample preparation	Analysis	Information obtained
SEM	-	Dehydrated	Overall real-time image	Detailed structure
CLSM	-	Hydrated, staining	Overall real-time image	Detailed structure
AFM	1 nm to 8µm	Not a specific sample preparation	Detailed real-time image	Very detailed structure

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DCPM	-	Placing colloidal beads	Video imaging together with determining the position of colloidal beads	Structural information
SAXS	10 to 1000 nm	Hydrated	Measuring the angle of the scattered photons	Structural information in the nanoscale
WAXS	Subnanometer scale	Hydrated	Measuring the angle of the scattered photons	Structural information in the subnanoscale
USAXS	Subnanometer scale	Hydrated	Measuring the angle of the scattered photons	Structural information in the subnanoscale
SANS	10 to 1000 nm	Hydrated	Measuring the angle of the scattered neutrons	Structural information in the nanoscale
3D bubble	-	Hydrated, but invasive staining	Real-time imaging together with a algorithm	Detailed structure
FTIRF	Nanometer scale	Hydrated	Data to compose the molecular composition	Molecular composition
NMR	Nanometer scale	Hydrated	Data to compose the molecular composition	Molecular composition
DSC	-	Hydrated	Difference in the amount of heat required to raise the temperature of a sample and a reference as a function of temperature	Detailed structure

Table 2 – a overview of all the methods discussed in this literature survey. It states the method, the details seen in the samples, the sample preparation, which kind of method it is and what information can be obtained.

All the methods have their advantages and disadvantages, but currently Scanning Electron Microscopy is still most used, because of the great details that can be obtained from the sample, but the samples need to be dehydrated. Confocal laser scanning microscopy looks like a better option, because the hydrogels are not dehydrated, but some sort of staining needs to be applied and this can be invasive.

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Atomic force microscopy might be a very detailed method, but it's too detailed to obtain overall structural information, it is not suited for mapping the pore sizes. Diffusing colloidal probe microscopy might be a method that can be used to obtain structural information on hydrogels, nonetheless, not a lot of research has been done on this and still beads need to be placed into the hydrogels, which might cause some damage.

The biggest advantage of the scattering methods is the ease of the sample preparation, because they can be placed into the machine without dehydration. The problem that will be encountered, is that the pore sizes could be too big to be detected, but other information on the structure can be obtained. SANS might be a better option than SAXS, because bigger molecules can be used.

The analytical methods NMR and FTIR are important to perform, to get an accurate view on the molecular structure of the hydrogel, but it's not suitable for obtaining the pore sizes or other structural information. 3D bubble is on the contrary a combination of analytical and fluorescence microscopy, which is very promising in literature. But at the same time, the staining can be invasive again. Differential Scanning Calorimetry has the same disadvantage, because again the samples need to be stained to be imaged by a confocal laser scanning microscope.

In conclusion, the scattering methods, in particular SAXS, are very promising in obtaining structural information on hydrogels. The method is quick and does not require sample dehydration, but some more experiment needs to be done to determine what information can be obtained next to pore sizes or distribution.

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