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Polysaccharide-based hydrogels with tunable composition as 3D cell culture systems

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ABSTRACT

Background: To date, cell cultures have been created either on 2-dimensional (2D) polystyrene surfaces or in 3-dimensional (3D) systems, which do not offer a controlled chemical composition, and which lack the soft environment encountered in vivo and the chemical stimuli that promote cell proliferation and allow complex cellular behavior. In this study, pectin-based hydrogels were developed and are proposed as versatile cell culture systems.
Methods: Pectin-based hydrogels were produced by internally crosslinking pectin with calcium carbonate at different initial pH, aiming to control crosslinking kinetics and degree. Additionally, glucose and glutamine were added as additives, and their effects on the viscoelastic properties of the hydrogels and on cell viability were investigated.
Results: Pectin hydrogels showed in high cell viability and shear-thinning behavior. Independently of hydrogel composition, an initial swelling was observed, followed by a low percentage of weight variation and a steady-state stage. The addition of glucose and glutamine to pectin-based hydrogels rendered higher cell viability up to 90%-98% after 1 hour of incubation, and these hydrogels were maintained for up to 7 days of culture, yet no effect on viscoelastic properties was detected.
Conclusions: Pectin-based hydrogels that offer tunable composition were developed successfully. They are envisioned as synthetic extracellular matrix (ECM) either to study complex cellular behaviors or to be applied as tissue engineering substitutes.

Keywords: Cell microenvironment, Injectable hydrogels, Pectin, Synthetic ECM, Tissue models

Introduction

Until recently, cell line and primary cell cultures have conventionally been created on 2-dimensional (2D) substrates. Although 2D cell culture systems have provided important mechanistic insights, they lack the 3-dimensional (3D) complexity of the in vivo environment, as provided by the morphological and topographical features of the extracellular matrix (ECM). Properties of the substrates, such as matrix stiffness and porosity, as well as surface topography, affect cellular responses including those directing cell fate (1-5). Three-dimensional substrates better mimic the natural in vivo setting compared with traditional 2D cell cultures in polystyrene.

The need for 3D environments for cell cultures is shared by different fields of application, such as in the development of constructs for regenerative medicine and in vitro models, a recent field of application extending the know-how derived from extensive research in regenerative medicine, to systems that mimic specific issues for different tissues (6, 7). In vitro models can be employed as fast screening tools for new therapeutics, selectively filtering active compounds before application in in vivo models (8, 9). Alternatively, they can be designed to study biological mechanisms underlying different pathologies (7, 10). Three-dimensional environments have been obtained with porous scaffolds (11, 12), as soft hydrogel (13-16) or microspheres (16-18).

In spite of continuous advances in the design of 3D microenvironments for tissue regeneration, these systems often do not match the biophysical and chemical ECM characteristics (19, 20).

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Biophysical characteristics include the mechanical forces between adhering cells and their surroundings, which differ in 2D and 3D environments. The polymeric or glass substrates for 2D in vitro cultures are usually stiffer compared with the soft hydrated ECM, which is more closely mimicked by hydrogels. First of all, hydrogels, as 3D soft environments, overcome spatial limitations and allow cell mobility and proliferation. In addition, it is highly desirable to provide the appropriate mechanical cues for the different physiological cell environments, with stiffness ranging from brain mimicking (0.1-1 kPa) to muscle-like and bone-like (8-10 and 25-40 kPa, respectively). The quest for hydrogels as a tunable mechanical environment is an advanced and recent topic of research, and an increasing range of hydrogels are reported to modify only the mechanical properties, independently from the biochemical cues (3, 20-27). Among the different substrates, polysaccharide hydrogels based on pectin can be produced with a range of stiffnesses by controlling the mechanism of crosslinking in mild conditions compatible with cell encapsulation (17, 28-30).

The issue of recreating the ECM's complex chemical environment might possibly be addressed by the tunable and controlled release of encapsulated bioactive agents from hydrogel matrixes (6, 9, 14, 19, 31-40), thus providing the necessary stimuli for cell migration, differentiation and production of neo-tissue growth or regeneration. The release of active molecules is limited by the difficulty of retaining their bioactivity and achieving in situ, optimal doses, gradients and timing.

Furthermore, the optimization of both density and 3D distribution of such molecules in the hydrogel structure needs to be tailored to the specific tissue to be regenerated. As an example, when delivered without stabilization, growth factors diffuse rapidly, undergo denaturation and consequently lose bioactivity under normal physiological conditions (30-32). The suboptimal release of growth factors, at high concentration or over a prolonged time, may lead to serious side effects, including the growth of tumors (41, 42). Thus, it is necessary to sustain bioactivity by developing controlled release systems (33, 34, 37-40) that can mimic the in vivo environment and the ECM's complex signaling.

In this work, we put forward the possibility of using simple, nonimmunogenic and non-patient-specific molecules, such as glucose (Glc) and glutamine (Gln), present in extracellular fluids and typically included in cell culture media, as universal additives for the production of 3D cell culture systems based on pectin hydrogels. These systems can represent a basic model to be implemented with more specific biomolecules for different purposes, and may be a cost-effective solution for in vitro 3D cell growth either for tissue engineering or for the development of in vitro tissue models.

Materials and methods

Materials

Pectin CU701 powder (lot 01011968) was kindly provided by Herbstreith & Fox (Neuenbürg, Germany). NaCl, $NaHCO_3$, $CaCO_3$ and Glc were supplied by Sigma Aldrich and used as received. Gln and all other reagents used for cell culture were purchased from Lonza, unless otherwise specified.

Preparation of the injectable hydrogels

Pectin solutions were obtained by dissolving 2.4% (w/v) pectin powders in 0.9% (w/v) NaCl, adjusting the pH with 23 mM $NaHCO_3$ as previously optimized (32). Pectin solutions were also prepared at the native pH – i.e., without $NaHCO_3$ – to be used as positive control (Pe0Ca). Pectin solutions were enriched with Glc or Gln, or with both additives in varying concentrations, up to 50 and 4 mM, respectively, prior to gel formation (Tab. I). The dose range was selected taking into account the typical concentration of Glc and Gln in cell culture media – i.e., 25 and 2 mM, respectively (38).

To form pectin hydrogels, 50 mM $CaCO_3$ was suspended in deionized water and sonicated at a frequency of 35 kHz, temperature 30°C for 60 minutes, then gently mixed with pectin solutions using a dual-syringe system (see supplementary figure 1, available online as supplementary material at www.artificial-organs.com – Procedure to form pectin hydrogels).

The gelling mixture was poured into 24-well plates to obtain size-controlled crosslinked samples (diameter 1 cm), and it was allowed to gel at 23°C ± 1°C for 10 minutes before adding the culture medium for incubation.

Characterization of the produced hydrogels

Measurements of pH

The pH of pectin solutions and hydrogels was measured with a Cyberscan pH 110219 pH meter (Eutech Instruments), using a specific electrode for soft hydrogels (Hamilton double pore slim). The pH measurements were performed on pectin solutions, before gelling, and on pectin hydrogels, immediately after preparation and after 1 hour of immersion in RPMI 1640 culture medium (Gibco).

Optical microanalysis of pectin hydrogels

The optical setup consisted of a standard Olympus BX61 microscope equipped with long working distance objectives. Sample images were acquired with a simple CCD camera (12 bit, 640 × 480 pixels, 7.4 × 7.4 µm in size; Pike Allied Vision).

For the optical measurements, a volume of about 0.6 mL of sample was confined between 2 optical glass slides. The final sample thickness was 1 mm, and in the observation plane, the sample area was 2 × 3 cm. The observation window was 860 × 645 µm in size. The gel sample was kept in conditions of controlled humidity to prevent evaporation.

A typical measurement consisted of a sequence of 200 images, acquired with a frame rate of 0.2 frames/s, an exposure time of 300 milliseconds, and a condenser numerical aperture (NA) = 0.3.

Rheological characterization

Rheological analysis was performed using an AR 1500ex rheometer (TA Instruments, Italy), equipped with parallel-plate

TABLE I - Hydrogel formulations (2% w/v pectin with 0.9% NaCl), crosslinked with 50 mM $CaCO_3$, employed for cell immobilization

Nomenclature	Composition			pH		
	$NaHCO_3$ (mM)	Glucose (mM)	Glutamine (mM)	Pectin solution	Hydrogel, as prepared	Hydrogels incubated in medium
Pe0Ca	0	-	-	3.10	5.63	7.35
PeCa	23	-	-	3.69	5.89	7.38
PeCa Glc	23	25.0	-	3.64	5.72	7.33
PeCa Gln	23	-	2.0	3.63	5.75	7.37
PeCa Glc/2	23	12.5	-	3.69	5.73	7.35
PeCa Gln/2	23	-	1.0	3.62	5.71	7.32
PeCa Glc/2 + Gln/2	23	12.5	1.0	3.64	5.93	7.39
PeCa Glc + Gln	23	25	2	3.65	5.91	7.36
PeCa 2Glc + 2Gln	23	50	4.0	3.68	5.88	7.31

The presence of 25 mM glucose and 2 mM glutamine is indicated as Glc and Gln, respectively, in the names of the samples, while Gln/2 and Gln/2 and 2Glc and 2Gln indicate half or double the said amounts, respectively. The pH and gel point of the different gel formulations are also reported.

geometry (diameter = 20 mm, working gap = 1,000 µm). Time sweeps (temperature 25°C, frequency 0.7 Hz, σ = 5 Pa) and frequency sweep (temperature 25°C, frequency 0.1-10 Hz, σ = 5 Pa) were conducted to evaluate the rheological parameters (storage modulus, tan δ and complex viscosity) of the pectin hydrogels.

Pectin hydrogel samples with an initial wet weight of 0.50 ± 0.05 g were immersed in culture medium (RPMI 1640) at room temperature for up to 24 hours. The percentage of weight variation was calculated [Eq. 1]:

$$\Delta W\% = \frac{W_t - W_0}{W_0} \times 100 \qquad \text{[Eq. 1]}$$

where W_t is the wet weight of gels at time point t and W_0 is the initial weight of the gels, measured immediately after their preparation.

Stability was determined by incubating pectin hydrogels (initial wet weight = 0.50 ± 0.05 g) in RPMI 1640 culture medium at 37°C and measuring their percentage weight loss (WL%) for up to 7 days [Eq. 2].

$$WL\% = \frac{W_0 - W_f}{W_f} \times 100 \qquad \text{[Eq. 2]}$$

where W_f is the final dry weight and W_0 is the initial dry weight of the samples.

Cell culture

A L929 cell line (mouse fibroblasts) purchased from the American Type Culture Collection (Rockville, MO, USA) was cultured in RPMI 1640 culture medium (pH 8.2), supplemented with 10% (v/v) fetal bovine serum, 1% L-glutamine, 1% sodium pyruvate and 1% penicillin/streptomycin. Cells were used within 7 to 9 passages after defrosting. Cells were incubated at 37°C in a 5% CO_2 atmosphere, until 70% confluence, and then trypsinized to be passaged.

Cell immobilization

L929 cells were immobilized in the different hydrogel formulations (Tab. I). The addition of Glc and Gln was modulated to evaluate a possible dose-dependent behavior.

L929 cells were suspended in pectin solutions with a density of 2.5×10^6 cells/mL. The cell-loaded solutions were crosslinked with 50 mM $CaCO_3$. Then, the crosslinking hydrogel (0.4 mL) was extruded from the dual-syringe system (see supplementary figure 1B, available online at www.artificial-organs.com – Procedure to form pectin hydrogels) into each well of a 24-well cell culture plate. The hydrogel was allowed to set for 5 minutes before the addition of 1 mL of culture medium, and the plates were incubated in controlled atmosphere, at 37°C in 5% CO_2.

Cell viability and metabolic activity

Trypan blue assay was performed after 1, 2, 4 and 24 hours of incubation. Cell pellets were recovered after dissolving the hydrogels with 1 mL of 0.9% (w/v) NaCl and centrifuging the samples at 1,300 rpm for 3 minutes. Then, the pellet was resuspended with 80 µL of trypan blue solution and 20 µL of fresh culture medium. Then 6.6 µL of the sample was drawn into a KOVA Slide[2] chamber (Glasstic Slide 10; KOVA International), and the cells were counted under an optical microscope (Olympus IX50). Cell viability (%) was expressed as percentage of the live cell number/the total cell number.

Metabolic activity was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After 4 and 24 hours of incubation of the cell-loaded gels, gels were dissolved, and the recovered cells were removed from the well with the gels and left overnight in new wells to proliferate. Then, MTT solution (5 mg MTT/1.5 mL phosphate-buffered

saline) was added, and the cells were incubated at 37°C. After 3 hours, the MTT solution was removed and replaced with 0.5 mL/well of isopropanol in 0.04 N HCl. The wells were stirred for 5 minutes until their color was uniform. Absorbance was evaluated at 595 nm. The analyses were performed in triplicate, using cells seeded on thermoresponsive tissue culture polystyrene (TCPS) as control.

To determine the viability of L929 cells during the first hour of immobilization, without dissolving the pectin hydrogels, a LIVE/DEAD® Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR, USA) was used. The assay was performed according to the manufacturer's instructions. Stained cells were examined under a Leica confocal laser scanning microscope (CLSM, model TCS SPII; Leica, Heidelberg, Germany) using a × 20 oil immersion objective. The excitation and emission wavelengths used for detecting calcein-AM were 494 nm and 517 nm, respectively. Ethidium homodimer-1 was excited at 527 nm, and its emission was monitored at 617 nm. Optical sections of 0.9 mm were collected from the complete thickness of the samples, and for each sample, images from 3 randomly selected positions were acquired. The resulting stacks of images were analyzed using Leica confocal software.

Statistical analysis

All measurements were made in triplicate, the results are presented as average weight variation and errors were calculated, with standard deviation. One-way analysis of variance (ANOVA) with Bonferroni post hoc analysis was used to determine any significant difference between the means of the samples. A difference was considered significant when the value of p was <0.05.

Results

Pectin hydrogels were obtained by internal gelation with calcium carbonate by mixing pectin and an aqueous suspension of the insoluble calcium (Ca^{2+}) salt (23) in a dual-syringe system (see supplementary figure 1, available online at www.artificial-organs.com – Procedure to form pectin hydrogels). The gelling mixture was extruded while the homogeneous dispersion was still behaving as a viscous liquid (<2 min from the beginning of the mixing), as determined by rheological measurements (data not shown). With this straightforward method, samples with reproducible size, shape and rheological properties were obtained.

Measurements of pH

The pH of pectin solutions increased during the crosslinking process for all of the formulations (Tab. I), reaching equilibrium after 1 hour of incubation in the culture medium. The addition of Glc and Gln did not alter this behavior.

Optical microanalysis of pectin hydrogels

$CaCO_3$ grain distribution and dimensions did not vary with aging (1, 4 and 24 hours). The use of pectin with higher pH led to the formation of hydrogels with a more homogeneous structure, having undissolved $CaCO_3$ grains equally distributed within the gel volume (Fig. 1A and B). Conversely, in Pe0Ca samples, the higher acidity of the pectin solution promoted a faster dissolution of the $CaCO_3$ powder, causing a marked CO_2 release. As shown in Figure 1C, CO_2 bubbles (with sizes varying from a few to several hundred microns) remained trapped in the hydrogel matrix because the increased stiffness of the gel prevented bubble motion and aggregation. Residual $CaCO_3$ grains with a wide range of polydispersity (30-100 µm) were also detected, but they were localized in limited areas of the hydrogels (roughly 20% to 30% of the analyzed samples), forming clusters of several hundred microns (Fig. 1D).

When the initial pH of pectin solutions increased (PeCa formulations, initial pH 3.69), the microstructure of the hydrogel was homogeneous within the whole sample volume, though $CaCO_3$ grains were not completely dissolved. The amount of CO_2 bubbles nucleated in these formulations was significantly lower than in the Pe0Ca hydrogels.

Rheological characterization

Both storage modulus G' and tan δ exhibited a frequency-independent behavior in the frequency ranges considered (Fig. 2). Tan δ was <1 for each hydrogel formulation, thus indicating the formation of solid-like gels, in agreement with previously reported results (28). Complex viscosity decreased for all hydrogels with increasing oscillation frequency, indicating a shear thinning behavior (Fig. 2B), typical of soft materials with viscoelastic properties (43, 44).

Storage modulus and complex viscosity were significantly higher for Pe0Ca than for hydrogels produced at increased pH of pectin solutions. No substantial effects over rheological properties were observed when comparing hydrogels enriched with Glc and Gln with PeCa hydrogels (Fig. 2), indicating the possibility of varying chemical composition while retaining the rheological properties.

The presence of a high density of L929 cells in PeCa and PeCa 2Glc + 2Gln (i.e., twice 25 mM Glc and twice 2 mM Gln) formulations did not induce significant changes in the rheological behavior of pectin hydrogels (Fig. 3).

Swelling and stability

Hydrogel swelling increased with time due to water absorption until equilibrium was reached after 4 hours of incubation, with average swelling in the range of 35%-70%, depending on the gel formulation. No clear trends could be found related to the amounts of additives (Glc and Gln) used in the gels (Fig. 4A).

All of the formulations tested lost between 8% to 20% their initial dry weight after 24 hours of incubation (Fig. 4B). After that, their weight was retained, and the hydrogels did not show any significant weight loss up to 7 days of incubation in the culture medium.

Cell immobilization and viability

To evaluate separately the contribution of each additive, L929 cell viability assessed in the hydrogels without additives (Pe0Ca and PeCa) was compared with that in hydrogels

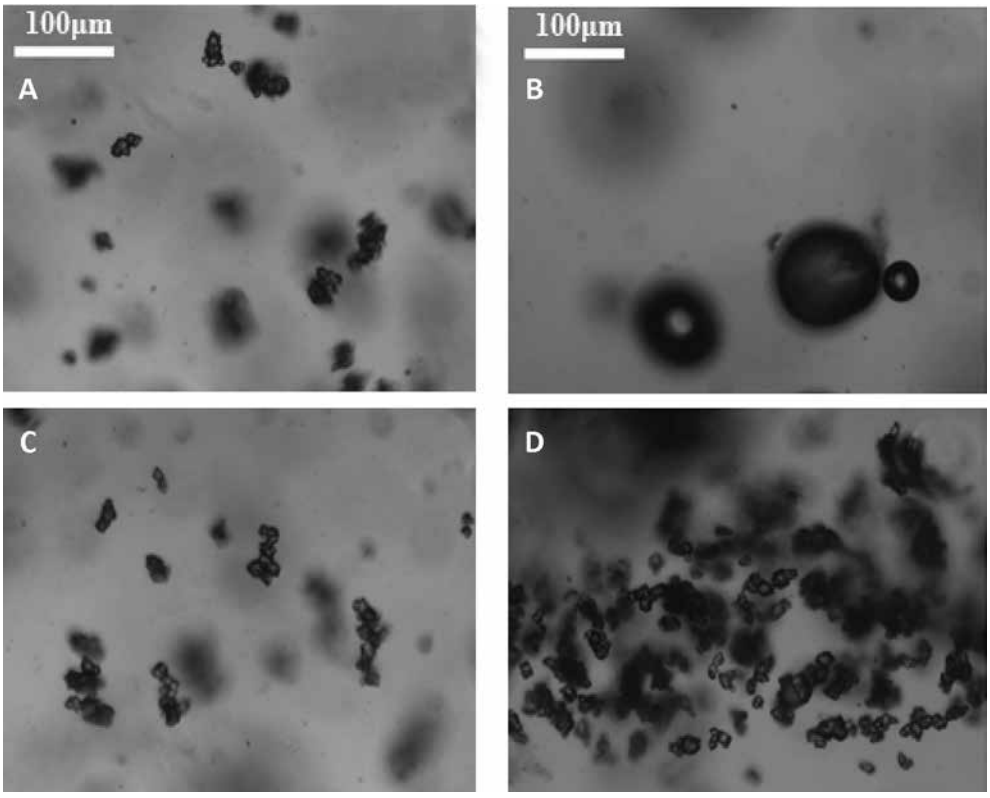

Fig. 1 - Images of PeCa solution (**A**, **B**) and pectin solution without $NaHCO_3$ (Pe0Ca) (**C**, **D**) hydrogels acquired 1 hour after preparation. In PeCa hydrogels, the distribution of the $CaCO_3$ grains is homogeneous, while in Pe0Ca, the acquired images show large areas without grains (**C**), the nucleation of CO_2 bubbles (**C**) and in a few zones, the formation of clusters (**D**) with a grain concentration much higher than in PeCa hydrogels.

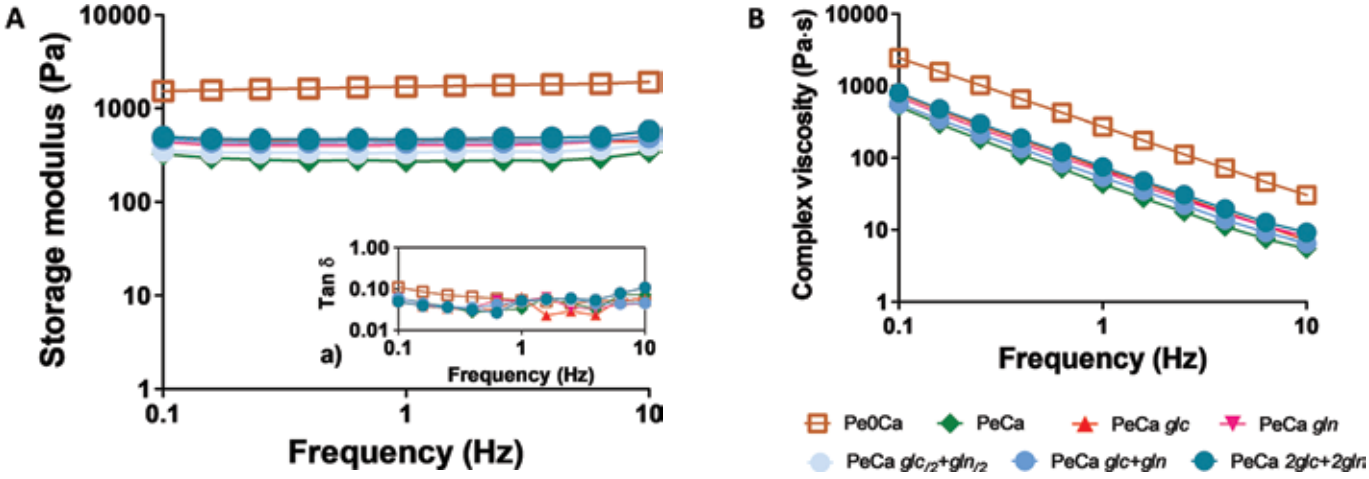

Fig. 2 - (**A**) Storage modulus, tan δ (inset (**A**)) and (**B**) complex viscosity of the hydrogel samples with different concentrations of glucose (glc) and glutamine (gln). www.amamanualofstyle.com/view/10.1093/jama/9780195176339.001.0001/med-9780195176339-div2-501.

enriched with either Glc or Gln. Poor L929 viability was observed in Pe0Ca samples from 1 hour up to 24 hours of incubation, due to the acidic pH of the hydrogels (Fig. 5 and Tab. I). For the other formulations, cell viability was retained in the range of 90%-98% starting from 2 hours of incubation onwards (Fig. 5A), probably due to the rapid neutralization of the pH and the increased availability of the culture medium nutrients by absorption in the swollen hydrogels. Metabolic activity (see supplementary figure 2, available online as supplementary material at www.artificial-organs.com – Absorbance values (related to cell viability) of the different gel formulations) was slightly higher at 4 and 24 hours for hydrogels containing Glc and Gln, being highest for PeCa 2Glc + 2Gln.

The results demonstrated that the presence of Glc and Gln improved cell viability in the short-term period, yet no differences were found in the long term, even when additives were used with high concentrations (Fig. 5B). Over time, the hydrogels equilibrate with the medium containing Glc and Gln, reducing the effect of the presence of the additives in the gels.

Confocal microscopy performed on the 3D hydrogels loaded with fibroblasts, confirmed that for all PeCa formulations,

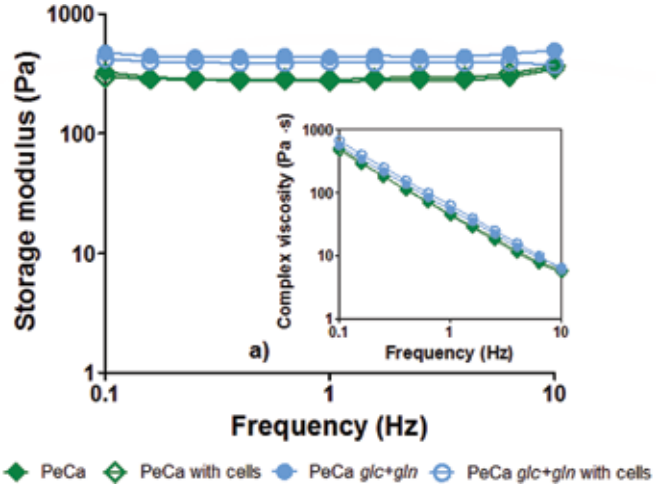

Fig. 3 - Storage modulus and complex viscosity (inset (**A**)) of the pectin solution hydrogel samples with and without immobilized L929 cells and with and without glucose (glc) and glutamine (gln) additives. www.amamanualofstyle.com/view/10.1093/jama/9780195176339.001.0001/med-9780195176339-div2-501.

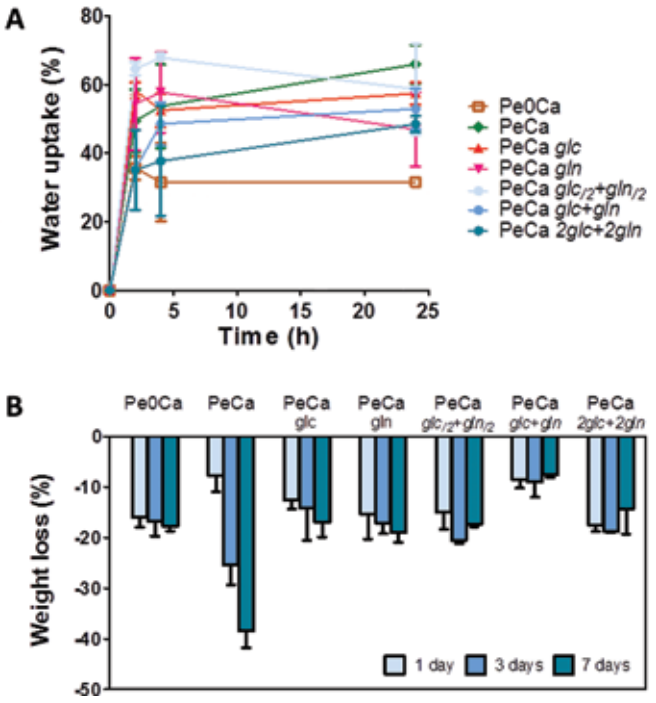

Fig. 4 - Swelling (**A**) and stability (**B**) of the gel formulations at different time points in culture medium.

most of the cells were viable (green fluorescence) and homogeneously dispersed in the 3D pectin network, and only a very few dead (red) cells were randomly distributed in the hydrogels (Fig. 5C and D).

Discussion

The current study proposed that a pectin-based hydrogel could provide a 3D platform for cell growth and proliferation. This platform could provide a base with a chemical composition that can be further tailored by the appropriate addition of different molecules, to serve as a synthetic ECM.

To achieve this, pectin hydrogels were obtained by internal gelation, mixing pectin and L929 cells with a poorly soluble calcium salt (i.e., calcium carbonate). The pH of pectin solutions was increased to reduce the release of the calcium (Ca^{2+}) ions involved in the gelation process, as well as to create a suitable environment for cell hosting. During crosslinking, only a thin superficial layer of the $CaCO_3$ grains was dissolved, as grains with comparable size and shape were measured before and after gelation (Fig. 1). Despite this minimal dissolution, the release of Ca^{2+} ions from the $CaCO_3$ grains was enough to produce solid-like gel structures in less than 3 minutes (data not shown). This kinetic profile allowed us to obtain a uniform 3D cell distribution within the PeCa hydrogels.

By controlling the kinetics of the crosslinking reaction, it was possible to obtain a homogeneous distribution of the cells along the 3 axes of the 3D hydrogel structure while retaining their viability upon extrusion (Fig. 5). As hydrogel crosslinking and cell immobilization occur in the same time period, the control of the kinetics of the hydrogel, and thus of the viscosity of the solutions during the gelling process, affects both the distribution of the cells within the hydrogel and cell viability. If the viscosity is too low, cell sedimentation may occur. In contrast, a viscous gel prevents a homogeneous dispersion of the cells and may induce shear stresses on the cell membrane when injected (45).

Pectin solutions with lower pH (Pe0Ca) promoted the rapid dissolution of $CaCO_3$ powders (Fig. 1C), resulting in the formation of highly crosslinked – albeit inhomogeneous – structures, as confirmed by the rheological analysis (Fig. 2) and swelling studies (Fig. 4A). However, they were not compatible with cell viability, due to their acid pH. Adjusting the pH with $NaHCO_3$ (PeCa formulations) was therefore essential to obtain 3D matrices suitable for cell immobilization. The increase of pH also showed an effect on hydrogel stiffness, resulting in a decrease of the rheological properties (G' and δ*) of the hydrogels (Fig. 2). It can be hypothesized that when cells are immobilized in a stiff gel matrix, the cell–cell interactions and signaling may be hampered and may be submitted to excessive shear stresses when injected (45, 46). The dependence of cell viability on pH and the rheological properties of the hydrogel was confirmed by the live vs. dead assay, where the cells immobilized in Pe0Ca hydrogels showed the lowest cell viability, as an effect of both the acidic pH and stiffer network structure.

In 3D networks, the viability of the immobilized cells can be reduced in the first hours of immobilization, due to the reduced perfusion and availability of nutrients. We therefore aimed to enrich the produced hydrogels with nonspecific additives to support the viability and metabolic activity of any cell type that could possibly be immobilized for tissue regeneration.

It has been generally accepted that Glc and Gln are the main carbon and energy sources of cell culture media (47-50). Glc consumption is one of the key indicators of cell metabolic activity, and it tightly correlates with the cell growth rate or the cell population dynamics. In 2D in vitro models, when the cell population increases rapidly (up to 3 or 4 days), a

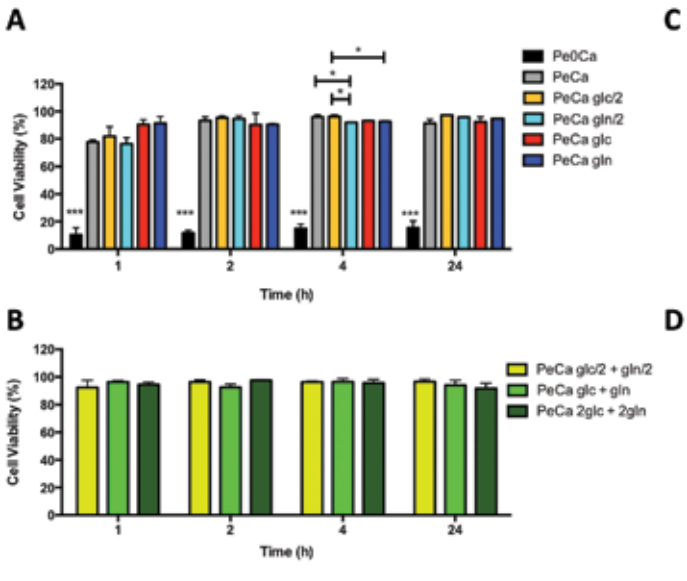

Fig. 5 - (**A**) Cell viability in the different gel formulations for up to 24 hours. One-way ANOVA was used (*p<0.05). All gels were significantly different from Pe0Ca (***p<0.001), and p values were <0.05 in PeCa vs. PeCa gln/2, PeCa glc/2 vs. PeCa gln/2 and PeCa glc/2 vs. PeCa gln at 4 hours of incubation. (**B**) Cell viability with increase of glutamine (gln) and glucose (glc) concentrations for up to 24 hours of immobilization. (**C**, **D**) Confocal laser scanning microscope (CLSM) images of viability of cells immobilized in hydrogels. Cells immobilized in PeCa (**C**) and PeCa glc + gln (**D**) hydrogel were stained at 1 hour. Sagittal sections of the samples are shown below and to the right of each panel. Scale bars are 200 µm.

large amount of Glc is consumed for cell proliferation (about 25 fmol/cell per day). Then, the cell population comes into steady phase, and Glc is mainly consumed for cell survival (48). Gln is naturally involved in a wide variety of processes, including energy production, nitrogen metabolism, ammonia detoxification and amino acid and protein metabolism (47), and under specific conditions, it can contribute significantly to cellular metabolism (51, 52).

According to the literature, high doses of Glc (about 20 mM) and Gln (2 mM) maintain cell viability and proliferation even in hypoxic conditions (47).

Glc and Gln appeared to maintain cell viability, even when used separately and at low concentrations (Fig. 5A). Over time, the swelling of pectin hydrogels (Fig. 4A) allows the nutrients of the culture medium that contains Glc and Gln to reach the cells within the gel, and the cell viability is kept over 90% for each formulation.

Regarding soft tissue regeneration and the development of in vitro tissue models, the soft hydrogels developed, enriched with Glc and Gln, represent a valid system, showing high viability of the immobilized cells, even in the short-term period.

Conclusions

In this work, pectin-based injectable hydrogels enriched with Glc and Gln were proposed as 3D microenvironments for cell encapsulation. The selected additives preserved the fibroblast cell viability even in the short-term period, which is known to be the most critical phase of cell immobilization. These additives can be used in different hydrogels to improve the viability of different cell types for the regeneration of diverse tissues. The possibility of tuning the composition of the pectin hydrogels, as shown in this work with nonspecific additives such as Glc and Gln, can be explored further and expanded to incremental levels of complexity for in vitro models or for tissue engineering, by addition of parameters to be implemented and controlled one by one.

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Disclosures

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