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# Design and development of a hybrid bioartificial water-induced shape memory polymeric material as integral component of a new means for the anastomosis of human hollow organs

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**KEYWORDS**

Bowel; Anastomosis; Hydrogels; Poly(vinyl alcohol); Acetylsalicylic acid

**RUNNUING TITLE**

A bioartificial water-induced shape memory polymeric material for the bowel anastomosis

**ABSTRACT**

A large number of pathologies require the resection of the bowel and anastomoses to rejoin the two remaining stumps to regain lumen patency. Various materials have been used to rejoin one bowel end to the other like catgut, stainless steel, and absorbable sutures. The actual method for anastomosis surgery uses entero-entero anastomosis (EEA) circular stapler with only a staple line. These methods can have some possible drawbacks, like intracellular fluid leakage and local inflammations. The aim of this study is to design and develop a novel bioartifical polymer with ring shape of polyvinyl alcohol

(PVA) and gelatin in ratio 80/20 (w/w) loaded both directly with acetylsalicylic acid and with nanoparticles incorporating the same drug to reduce possible local inflammation also at prolonged times. A physical method (8 cycles freezing/thawing) was used to obtain a crosslinked bioartificial shape memory ring.

Mechanical analysis showed a storage modulus having a comparable value with that of human colon. HPLC analysis pointed out a sustained and prolonged release of the anti-inflammatory drug for a suitable effect both immediately after anastomosis surgery and during healing period. Cell culture tests indicated the cytocompatibility of the bioartificial device. A shape memory of the hydrogel prepared in ring form was observed at 37 °C after immersion in water. These bioartificial devices can represent a new approach to serve as a multifunctional anastomotic ring .

## 1 INTRODUCTION

Numerous surgical conditions require the resection of bowel segments and the creation of reliable anastomoses [1]. A wide variety of suture materials have been used to form hand-sewn anastomoses. Bowel anastomoses are common procedures in both elective and emergency general surgery. The selected anastomotic technique depends on site of anastomosis, bowel caliber and characteristics of underlying disease process. The key to a successful anastomosis is the accurate union of two viable bowel ends with complete avoidance of tension. Three of the most significant complications related to intestinal anastomosis are dehiscence, leakage, and late stenosis. Indeed, breakdown of an anastomosis is associated with considerable perioperative morbidity and mortality. Over the past decades, a large number of materials have been used to rejoin one bowel

end to the other, i.e. naturally absorbing compression rings (Valtrac biofragmentable anastomotic ring BAR), non absorbing compression rings (Murphy compression button), AKA-2 device, compression anastomosis clip (CAC) and compression anastomosis ring (CAR) [2-7]. Actually, the surgical community uses gladly entero-entero (EEA) circular stapler with only a staple line. This method presents some limitations, including intracellular fluid leakage and local inflammations. Generally, the presence of a material can activate inflammatory reaction in the human body and surgical sutures are not excluded by this observation. Classical surgical materials, like silk, can generate inflammatory reaction that can persist for weeks after surgery. Materials such as polypropylene, catgut, and polyglycolic acid have a milder response. For this reason, the use of one of the more biocompatible materials, as the PVA, can lead to a reduction of inflammatory reactions. Particularly, PVA hydrogels proved to be excellent materials for biomedical application due to their chemical and mechanical properties [8-12]. Moreover, the addition of gelatin, a biological polymer and thus intrinsically biocompatible, to the PVA could further improve the tissue response. The combination of synthetic polymeric materials with natural macromolecules has been of great interest to biomaterial scientist in the recent past [13]. These systems have attracting characteristics for the realization of new materials for biomedical applications. It is well known that PVA hydrogels do not support cell spreading and adhesion. For this reason, gelatin was added to the PVA solution, in different ratios, obtaining a bioartificial polymeric material with the aim to promote cell adhesion and growth [14]. In this study, we propose for the first time the use of a bioartificial material to produce a shape memory ring for intestinal anastomosis. PVA/gelatin water solution at different

composition were prepared and then were subjected to crosslinking using the freeze-thawing method (Nambu method) [15-17]. In view of application as anastomosis rings to reduce the local inflammation, PVA/gelatin hydrogels were loaded with acetylsalicylic acid, by means both direct adsorption from drug solution and nanoparticles loaded in turn with acetylsalicylic acid. Finally, all the systems prepared in this work were characterized in terms of physico-chemical and mechanical properties, water shape memory behavior, drug release and cytocompatibility.

## 2 MATERIALS AND METHODS

### 2.1 Materials

Poly(vinyl alcohol) used in this work has a molecular weight of 85,000-124,000, with a degree of hydrolysis of 99% (PVA, Sigma Aldrich). Gelatine A from porcine skin (Gel, Sigma Aldrich) was used as received to obtain bioartificial materials. Acetylsalicylic acid (Asa, Sigma Aldrich), also known by trade name Aspirin®, was used to reduce local inflammation.

### 2.2 Preparation of PVA hydrogels by chemical crosslinking

The PVA hydrogels were prepared using two different ways: chemical crosslinking by means glutaraldehyde addition (GTA, Sigma Aldrich), and physical crosslinking [15, 16]. For the first method, 4 ml of glutaraldehyde (GTA, 25 wt% aqueous solution) were added to 100 ml PVA aqueous solution (15 wt%) previously modified by adding hydrochloric acid to obtain a solution at pH 4. The solution was stirred at 500 rpm and poured in glass capsules. The chemical crosslinked reaction was conducted for 36 h at

room temperature (25°C). Afterwards the samples were subjected to repeated washings with MilliQ water to remove unreacted GTA. Then the samples (PVA/GTA) were dried for 48 h at room temperature and finally the samples were placed in an oven at 37°C for 6 days.

### 2.3 Preparation of PVA/Gel hydrogels by physical crosslinking

For the preparation of PVA/Gel hydrogels, a predefined amount of gelatin was added to a PVA aqueous solution (15% w/v) in order to obtain PVA/gelatin (PVA/Gel) blends at different composition 100/0, 80/20, 70/30, 60/40 (w/w). The blends were dispensed in Petri dishes and freezing- thawing method was used. In particular, the samples were placed in freezer at -20°C overnight and successively subjected to 8 cycles of thawing for 1 h (+20°C) and freezing for 1 h (-20°C).

### 2.4 Preparation of PVA/Gel hydrogels loaded with Asa and Asa-Nps

Asa was dissolved in MilliQ water at the maximum water solubility (3 mg/ml) for 1 h at 30°C under magnetic stirring (500 rpm). To favor the molecular interaction between drug molecules and polymer matrix Asa was added directly in the PVA/Gel solution at 80/20 ratio [18]. The loading of drug was carried out on selected PVA/Gel 80/20 hydrogels on the basis of preliminary mechanical results (Table 2).

A PVA/Gel/Asa solution was prepared in autoclave for 1h at 120°C. Nanoparticles (Nps) based on a butyl methacrylate, poly(ethylene glycol) methyl ether methacrylate and 2-

(dimethylamino)ethyl methacrylate (PBMA-(PEG)MEMA-PDMAEMA) were prepared as reported in a previous article [19] and subsequently loaded with Asa. Before drug loading, Nps (5 mg) were allowed to swell in MilliQ water for 24 h at room temperature. Then a predefined volume of Asa solution at 0.5 mg/ml was added to Nps, stirred vigorously by vortex for 15 min and then centrifuged for 15 minutes at 14,000 rpm to obtain Asa-Nps.

Afterwards, Asa-Nps were incorporated into PVA/Gel/Asa solution; an amount of 10 mg of Asa-Nps was added to 10 mL of PVA/Gel/Asa solution. The final solution was poured into ring shape moulds and placed at -20°C overnight. All the samples underwent eight cycles of freezing (- 20°C) and thawing (+ 20°C) of 1 h for each cycle to obtain bioartificial hydrogels.

## 2.5 Characterization of PVA-based hydrogels

### 2.5.1 Physico-chemical analysis of PVA/Gel hydrogels

To evaluate the chemical distribution of hydrogel components, FTIR Chemical Imaging analysis was carried out. PVA/Gel hydrogels having a thickness of 1-2 mm were prepared and analyzed by FTIR using Transmittance Mode. Spectral images were acquired in transmission and in µATR mode using the infrared imaging system Spotlight 300 (Perkin Elmer). The spectral resolution was 4 $cm^{-1}$. The spatial resolution was 100x100 µm in µATR mode and 6.25 µm in transmission. Background scans were obtained from a region of no sample. IR images were acquired with a liquid nitrogen cooled mercury cadmium telluride line detector composed of 16 pixel elements. Each

absorbance spectrum composing the IR images, and resulting of 16 scans, was recorded for each pixel in the μATR mode using the Spotlight software. Spectra were collected by touching the ATR objective on the sample and collecting the spectrum generated from the surface layer of the sample. The Spotlight software used for the acquisition was also used to pre-process the spectra. IR spectral images were produced by using the absorbance in a given frequency range, 4,000-720 $cm^{-1}$. Spectra contained in the spectral images were analyzed using a compare correlation image. The obtained correlation map indicates the areas of an image where the spectra are most similar to a reference spectrum [20].

2.5.2 Water weight loss and swelling degree

The hydrogel samples were dried at 37°C for 7 days in an oven until reaching constant weight ($W_d$) [21,22]. Dry hydrogels were weighed every 12 h for 7 days. The degree of water loss was calculated using the following equation (Eq. 1):

$$DWL = \frac{Wi - Wd}{Wi} \cdot 100$$

Where:

$W_i$ = weight of the initial sample;

$W_d$ = weight of dry sample.

After hydrogel samples were weighed and then immersed in MilliQ water at 25°C for 7 days to reach equilibrium. The swollen samples were weighed every 12 h for 7 days. The equilibrium degree of swelling (EDS) was calculated using the following equation (Eq. 2):

$$EDS = \frac{Ww - Wd}{Wd} \cdot 100$$

Where:

$W_d$ = weight of dry sample;

$W_w$ = weight of wet sample.

Data from each sample were calculated as average of three measurements.

2.5.3 Mechanical characterization of PVA-based hydrogels

Mechanical properties for each hydrogel formulation, rectangular specimens 10 x 8 x 2 mm, were evaluated by DMA 8000 (Perkin Elmer) in compression mode at a frequency of 1 Hz and constant temperature of 37°C. The compression mode analysis was carried out at 37°C both in dry and in wet condition for the whole duration of the test (fluid bath configuration). Tests were carried out in triplicate.

2.5.4 Release test

High-performance liquid chromatography (HPLC) was used to determine the Asa amount in aqueous solution and corresponding release kinetic [23,24]. A Perkin-Elmer Series 200 instrument provided with UV Detector and Autosampler was used. Quantification of Asa was achieved using a column Phenomenex Synergi Hydro C18 and the following method: ACN/$H_2O$ 70/30 (v/v) as mobile phase, flux of 1 ml/min, injected volume of 100 µL; detector wavelength of 210 nm.

2.5.5 Water-induced shape memory behavior

Preliminary water-induced shape memory behavior was investigated for the PVA/Gel 80/20 (SM-PVA/Gel 80/20) hydrogel having a ring shape. The shape-memory behavior was examined in a different way compared to quantitative manner, which use the bending test [25-26]. The SM-PVA/Gel 80/20 sample was pre-deformed in an oven at T=37°, below its glass transition temperature [27-28], until a constant weight was reached, under constant constraint and followed by cooling to a room temperature (T=25°C) where the temporary shape was fixed. Then, the deformed sample was allowed to swell in MilliQ water to recover the initial shape. After 7 days, internal and external radius and thickness of hydrogel ring were measured and the volume-swelling ratio was calculate to evaluate the water induced shape memory behavior.

2.5.6 Sterilization and cytotoxicity evaluation

*Sterilization procedure*

Sterilization was performed onto circular samples (1 cm ø) of each hydrogel before cell culture tests. The samples were dried in oven at 37°C for 4 days and afterwards the samples were conditioned in MilliQ water at room temperature for 4 days. Then, samples were immersed in sterilisation medium (70% EtOH aqueous solution) for 1 h by changing the medium every 15 min. Afterwards, the samples were dried and placed under UV light for 15 min on each sample side to complete the sterilization procedure.

*Seeding and culture of hDFs on hydrogels*

Three hydrogels for each series were placed in wells of a 24-well plate and preconditioned over night in complete DMEM medium. After medium removal, $10^4$ human dermal fibroblasts (hDFs) at passage VI were resuspended in 50 µl of complete DMEM medium (Life Technologies) supplemented with 10% fetal bovine serum (Euroclone) and seeded in drops on the surface of each hydrogel. After 4 h, time required for cell adhesion, 700 µl of complete medium were added to cover the materials, then the 24-well plate was placed in humidified incubator with 5% $CO_2$ at 37°C. Cells seeded on the hydrogels were maintained in culture for 8 days and the medium was replaced every 3 days.

*Cell viability on hydrogels*

Cellularized hydrogels were washed briefly in PBS and cell viability was determined by staining with 4µmol/l Calcein-AM (Sigma-Aldrich, USA) for 30 minute at 37°C and then analyzed by confocal microscopy (Carl Zeiss Laser Scanning System LSM 510). The staining was repeated on the same materials at 1, 3, 5 and 8 days of culture. The analysis includes also cells adherent on the bottom of the wells.

*Cell adhesion and proliferation on hydrogels*

Cell adhesion was evaluated after 24 h of culture using CellTiter-Blue® assay (Promega, USA), a metabolic assay used to assess the redox activity of living cells by conversion of resazurin dye into the fluorescent product resorufin. Fluorescence

measured in the medium is proportional to the number of living cells. Concomitantly with seeding on PVA scaffolds, hDFs were also plated in a 24 well plate to create a 2D calibration curve with increasing number of cells (0, 2500, 5000, 10000, 20000, 40000 cells/well). After 24 h of culture resazurin was added to the medium of each well at a 1:10 ratio and incubated for 7 h at 37°C. Then supernatants were collected and fluorescence emission read at 590 nm with a multi-plate reader (GloMax, Promega, USA). Values of the calibration curve were used to compare the fluorescence measured in cells seeded on the hydrogels in order to calculate the percentage of adhesion. To evaluate proliferation on hydrogels CellTiter-Blue assay was repeated on cellularized materials at 3, 5, 8 days after seeding and the fluorescence obtained normalized on the initial value recorded at 24 h. After each test, cellularized hydrogels were washed twice with PBS and then fresh medium was added to continue the culture.

## 3 RESULTS

### 3.1 FT-IR

In Fig.1 (a) FT-IR spectrum of PVA/Gel (80/20 w/w) hydrogel obtained by physical method is reported. Main peaks related to two components were detected: the large band between 3700 and 3000 $cm^{-1}$ corresponding to O-H stretching and N-H stretching [29], the band at 1660-1600 $cm^{-1}$ referred to C=O stretching (Amide I), and the peaks between 1580 and 1520 $cm^{-1}$ of N-H stretching and C=O (Amide II). Moreover, C-H stretching of alkyl groups between 2700 and 3000 $cm^{-1}$, and the peak between 1150

and 1050 $cm^{-1}$ referred to C-OH stretching of hydroxyl group were also present. Fig.1 (b) shows the correlation map respect to FT-IR medium spectrum.

### 3.2 Water weight loss and equilibrium swelling degree

Water weight loss degree (WLD) and equilibrium swelling degree (ESD) for pure PVA hydrogels obtained by means both chemical and freezing-thawing method PVA/Gel hydrogels, before and after loading with Nps, are reported in Table 1. The data were obtained from samples dried in an oven at 37°C until reaching constant weight. The samples were weighed every 12 h for 6 days, with the exception of the first day where the intervals were: 10 min, 20 min, 30 min, 1h, 2h and 4h. From data reported in Table 1, an evident difference between the WLD at 6 days of PVA/GTA hydrogels (71.3%) and that of physical PVA hydrogels (85.9%) was registered. In Fig. 2 the trend of WLD versus time for hydrogels is reported. From a kinetic point of view, the maximum value of WLD was reached for physical PVA hydrogel after 24 h while the trend of WLD for PVA/GTA hydrogel is slower and prolonged. Unlike PVA/GTA hydrogel, in all other cases the maximum of weight loss occurs around 24 h and reaches the steady state at about 48 h (Fig. 2). The initial rate of water weight loss for PVA hydrogel in the presence of Nps seems to decrease but at the equilibrium its WLD value (85.3%) is similar to that of unloaded hydrogel PVA (85.9%). On the contrary, the effect of the presence of gelatin in the hydrogel on WLD is slightly more evident. As shown in Table 1 and Fig. 2, the rate of weight loss is decreased within 24 h with a decrease of about 3 percentage points both in the unloaded PVA/Gel sample and in Nps-loaded PVA/Gel hydrogel respect to their corresponding PVA hydrogels.

The ESD values measured for all analyzed hydrogels are shown in Table 1 and Fig. 3. The data were obtained after immersion of the samples in a MilliQ water bath at room temperature for about 1 week. The samples were weighed every 12 hours. It is possible to observe that PVA/GTA hydrogel reabsorbed about the same water amount (70.2%) lost in the loss weight tests, with a slow water recovery trend. Higher water absorption values for physical PVA hydrogels were registered (159.8%), in addition the presence of Nps did not modify the ESD values showing only an initial weak slowing of water absorption kinetic (Fig. 3). Elevated swelling values for PVA/Gel hydrogels, higher than 200%, were registered. Also in this case, the presence of Nps induce a decrease of water adsorption rate, however the final value of ESD for PVA/Gel/Nps (215.6%) hydrogel settles at the same level of unloaded PVA/Gel hydrogel (214%).

**3.3 Mechanical analysis and stability test**

The values of storage modulus (E') and loss modulus (E'') obtained for different hydrogel formulations using compression mode in dry state were reported in Table 2- 3. By comparison storage modulus (E') of PVA crosslinked with GTA and that of freezing-thawing PVA [27], it can be observed a higher E' value for PVA/GTA (5.7 MPa). In the case of bioartificial PVA/Gel hydrogels obtained by physical method, the presence of gelatin induces a reduction of E' value. E' values decrease with the increase of gelatin content, as evidenced by the comparison of the values obtained for hydrogels from PVA/Gel 100/0 to 60/40 (Table 2). The addition of polymeric nanoparticles leads to a light increase in the E' and E'' moduli, even if the correlation with the hydrogel composition is maintained as shown for hydrogels without Nps (Table 2). On the contrary the direct loading of Asa into the PVA/Gel hydrogel induces a slight reduction of

E’ and E’’, such reduction seems even slightly increased when the direct incorporation of drug is combined with Nps loading (Table 3).

### 3.4 Water-induced shape memory behavior

On the basis of swelling and mechanical results, we decided to evaluate the water-induced shape memory behavior for the PVA/Gel 80/20 hydrogel prepared with a ring shape. The maximum volume expansion of the ring hydrogel was measured using a digital caliper. Size measurements (external and internal diameter and thickness) were carried out on ring hydrogel after preparation phase (initial size) and after drying with a constant force in oven at 37°C until reaching a constant weight (dried sample). Then, deformed ring hydrogel was allowed to swell in MilliQ water for 7 days to recover the initial shape (swollen sample) (Fig.4). Size measurements of ring hydrogel obtained under three different conditions are reported in Fig 5, showing the maintenance of ring-shape regardless of state. .To had better understand the behavior of water induced shape memory in SM-PVA/Gel 80/20, the volume swelling degree was evaluated. By comparing the volumes of ring hydrogel at the initial condition ($V_i$ 5791 $mm^3$), after drying ( $V_d$ 1635 $mm^3$) and after swelling ($V_w$ 4597 $mm^3$), it can be seen an elevated volume-recovering after swelling of about 80 % ($V_w$ /$V_i$ %) respect to initial volume, while the volume expansion of the ring hydrogel from dried state to swollen state $V_w/V_d$ % reaches even a value of about 280%.

### 3.5 Drug encapsulation efficiency and drug-release kinetics

To evaluate both the encapsulation efficiency of Nps loaded with different drug concentrations (0.1-0.3 mg/ml) and their drug-release kinetics, HPLC analysis was performed. In Fig.6 (a) the drug encapsulation versus time curves show for all concentrations the reaching of the encapsulation maximum after about two days. In Fig.6 (b) the encapsulation efficiency (EE%) measured at three days for all samples was reported. In details, the nanoparticles loaded with 0.5 mg/ml Asa pointed out the highest encapsulation efficiency (44.54 %). For this reason, Nps loaded with 0.5 mg/ml of drug were chosen to carry out drug release tests. In Fig.7 (b), drug release percentage at equilibrium for Asa-Nps, for PVA/Gel hydrogel loaded with Asa-Nps and for PVA/Gel hydrogel with combined loading (Asa and Asa-Nps) was reported.

The combined release was evaluated to provide a more immediate and substained drug release thanks to direct drug loading, in order to contrast rapidly the anastomosis inflammation process, and at the same time by means the release from Nps a more controlled and prolonged drug release to reduce chronic inflammation.

In Fig.7 (a) the delivery kinetics curves obtained by directly loaded hydrogels and by hydrogels with combined loading are reported. It is possible to notice that the release efficiency is rather high for both samples, and as expected a greater amount of drug is released from the hydrogels with combined loading.

### 3.6 Functional test

To evaluate the resistance of a PVA/Gel 80/20 hydrogel obtained by physical method when subjected to stapling. A sample after swelling was stapled and its surface was analyzed using an optical microscope. In Fig.8 (a) the absence of cracks on the sample

surface and an excellent adhesion between the staple and the hydrogel without structure deformation were observed. In Fig 8 (b) the image of a PVA hydrogel crosslinked with GTA after stapling was reported; it is evident by the comparison with the physical hydrogel the presence of fractures and a clear separation between the staple and the PVA/GTA hydrogel that makes it unsuitable for anastomotic application.

### 3.7 Cytocompatibility tests

Preliminary studies of cytocompatibility on the hydrogels examined in this study were performed. Human fibroblasts were seeded onto hydrogels placed in 24-well plates and the confocal microscopy evaluation allowed to point out the presence of viable and elongated cells attached at the bottom of the wells in the presence of all examined samples (Fig.9 (a)). However, in the case of pure PVA hydrogels cell adhesion at 24 h was lower than that registered for PVA/Gel hydrogels also in the presence of Asa-Nps (and/or pure Asa) (Fig.9 (b)). This result suggests that the presence of gelatin uniformly distributed on the surface favors human fibroblast adhesion on the PVA-based hydrogels. Cell proliferation tests, obtained by measuring fluorescence normalized on the initial value recorded at 24 h, demonstrated the ability of cells to proliferate on all samples analyzed. In Fig.9 (c) a comparison between the fluorescence measured after 3 days and that obtained after 24 hour was illustrated; cells increase respect to 24 hours was registered for all samples with a value higher than 2 fold in the case of bioartificial hydrogel with loading combined.

## 4. Discussion

Here we demonstrated the good physical, mechanical and chemical properties of an anti-inflammatory drug delivery biartificial hydrogel for anastomosis application. The hydrogel system developed in this study aimed at combining a better compliance to bowel tissue respect to standard stapler method currently in use along with a local and controlled anti-inflammatory action at the injury site. The use of PVA-based hydrogels represents an unconventional approach for the realization of compression rings that currently are obtained starting from polyester bioresorbable [30] in literature several reasons. First, the hydrogel obtained by freeze-thaw method is to be considered particularly attractive for biomedical application because the preparation ease the un-necessity of complex fabrication apparatus and the absence of potential toxic agents. In addition the PVA polymer *per se* and after processing into physical hydrogels is known for its biocompatibility and capability of blending with a large spectrum of natural polymers (gelatin, enzymes, chitosan et al.) without altering the formation of an homogeneous hydrogel structure [31-33]. In this regard, chemical imaging technique used to investigate hydrophilicity and molecular interactions between material components indicated a good chemical homogeneity of the bioartificial hydrogel components, as evidenced by correlation value close to 1 (Fig.1 (b)).

The evaluation of physico properties, by water weight loss degree (WLD) and equilibrium swelling degree (EDS), showed an evident difference between the WLD at 6 days of PVA/GTA hydrogels (71.3%) and that of physical PVA hydrogels (85.9%). The maximum value of WLD was reached for physical PVA hydrogel after 24 h while the trend of WLD for PVA/GTA hydrogel is slower and prolonged. This result is probably due to the fact that GTA creates more stable and fixed bonds with the PVA chains respect to

hydrogen bonds due to hydroxyl group interactions occurring in the physical hydrogel, thus limiting the water evaporation. Unlike PVA/GTA hydrogel, in all other cases the maximum of weight loss occurs around 24 h and reaches the steady state at about 48 h (Fig. 2)

For the ESD value the PVA/GTA hydrogel reabsorbed about the same water amount (70.2%) lost in the loss weight tests, confirming the stability of the structure due to chemical crosslinking and its poor hydrophilicity. Elevated swelling values for freeze-thaw PVA/Gel hydrogels (214%) were registered. From these data, it can be observed that the presence of gelatin further increases the hydrophilicity characteristics of PVA-based hydrogels (159.8%). In addition the loading with Nps does not influence the ability of swelling for hydrogels (215.6%); even if Nps have a highly crosslinked structure, however their dimensional impact can be considered almost negligible respect to the hydrogel macrostructure.

Mechanical analysis showed a higher E' value for PVA/GTA (5.7 MPa), due to the formation of covalent bonds among PVA hydroxyl groups and GTA that increases the rigidity of the hydrogel. This result is in agreement with the swelling behavior obtained for the same hydrogels. For the PVA/Gel hydrogel the amorphous characteristics of gelatin macromolecules seem to have an influence on E' and E'' by lowering the rigidity of the hydrogels. Importantly, mechanical analysis showed a storage modulus of hydrogel system in good agreement with the value reported for human colon [34].

On the basis of the results (Table 2), we considered 80/20 ratio as the more suitable composition for the application requirements providing an adequate mechanical support to reduce anastomosis leakages and at the same time offering a substantial protein

content. The presence of a protein into the material is of great importance to allow the cell adhesion considering the poor ability of cells to adhere onto hydrogels due to their high interfacial energy.

To evaluate both the encapsulation efficiency of Nps loaded with different drug concentrations (0.1-0.3 mg/ml) and their drug-release kinetics, HPLC analysis was performed in the absorption and release media. The nanoparticles loaded with 0.5 mg/ml Asa pointed out the highest encapsulation efficiency (44.54 %). For this reason, Nps loaded with 0.5 mg/ml of drug were chosen to carry out drug release tests.

A rapid release of Asa at the initial phase and a subsequent prolonged delivery was observed allowing to obtain a drug release both immediately after the anastomosis surgery and during the healing period.

Importantly, functional tests like to stapling the sample, in order to simulate surgical procedure, demonstrated the absence of cracks on the PVA/Gel 80/20 sample and an excellent adhesion between the staple and the hydrogel without structure deformation.

Test of water-induced shape memory behavior were conducted in a sample of PVA/Gel 80/20 with a ring shape. After being immersed into water for several days at 25°C, a noticeable expansion in volume was obtained along with the shape change to recover the initial shape. Particularly, a volume swelling degree of about 80% was found, more that previous studies [25], maybe due to physical crosslinking. In  a preliminary phase the presence of water-induced shape memory behavior in physiological condition was evelauted. For this raison, the temperature of 37°C as temperature of pre-deformed shape was selected. The recovering ring shape was evaluate after 7 days, considering

that usually the healing process occurs in this time period, as reported in literature. [35-36]

Preliminary cell culture tests using human dermal fibroblasts were performed. PVA hydrogels cell adhesion at 24 h was lower than that registered for PVA/Gel hydrogels also in the presence of Asa-Nps and/or pure Asa. This result suggests that the presence of gelatin uniformly distributed on the surface favors human fibroblast adhesion on the PVA-based hydrogels. Cell proliferation tests demonstrated the ability of cells to proliferate on all samples analyzed suggesting the cytocompatibility of the hydrogel systems.

## 5. Conclusion

In this work, a bioartificial hydrogel was developed for the first time to reduce the local inflammation occurring after anastomosis surgery on the human hollow organs. The current method for anastomosis surgery uses entero-entero anastomosis (EEA) circular stapler with only a staple line. In order to reduce the drawbacks of this method, a new bioartificial polymeric material with ring shape and hydrogel structure was proposed. Bioartificial hydrogels of PVA and gelatin (PVA/Gel 80/20, w/w) with both acetylsalicylic acid and nanoparticles loaded with the same dug were prepared and characterized. The samples were obtained in form of gel-like materials using physical method (freezing/thawing). Gelatin was added to improve fibroblast adhesion, which is an important step for extracellular matrix synthesis and complete sealing. In addition, to reduce local inflammation, hydrogels were modified with acetylsalicylic acid (Asa) both

by direct loading and nanoparticles (Asa-Nps) for a more controlled release. These materials showed good mechanical properties, ability to release an antinflammatory drug, biocompatibility and a relevant water induced shape memory behavior.

The results obtained from various analyses, in addition to other advantages including ease of preparation and low cost, suggest that the hydrogels developed in this study have the potential to serve as anastomotic ring devices.