SUPPORTING SCIENTIFIC INFORMATION ON POLYACRYLAMIDE (PAAG) HYDROGEL

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CHAPTER 1

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1/ GELIPERM CASE: 3% PAAG HYDROGEL FOR TOPICAL WOUND CARE

KNAPP et al.
Clinical experiences with a new gel-like wound dressing after skin transplantation.
Aktuelle Traumatol. 1984 Dec;14(6):275-81

KICKHÖFEN et al.
Chemical and physical properties of a hydrogel wound dressing.
Biomaterials. 1986 Jan;7(1):67-72

SATTLER et al.
Wound management with split skin flaps--donor sites. Covering with the moist gel Geliperm.
Fortschr Med. 1990 Feb 20;108(5):94-6

2/ PAAG HYDROGEL FOR TOPICAL DRUG DELIVERY SYSTEM

JAISWAL et al.
Polycaprolactone diacrylate crosslinked biodegradable semi-interpenetrating networks ***of polyacrylamide and gelatin for controlled drug delivery.***
Biomed Mater. 2010 Dec;5(6):065014

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Aktuelle Traumatol. 1984 Dec;14(6):275-81.

Clinical experiences with a new gel-like wound dressing after skin transplantation.

Knapp U, Rahn HD, Schauwecker F.

Abstract

Free split skin grafting is now widely practised on a routine basis in accident surgery. All conventional wound dressing methods have considerable drawbacks, and this is one of the reasons why successful skin grafting is still an unsafe procedure even under the very best of conditions and quite often resembles an experiment more than a scientific procedure, with an unpredictable outcome. Today, however, we can command over a wound dressing, using the polyacrylamide agar hydrogel developed by the Max Planck Institute of Immunobiology at Freiburg and which is being marketed under the trade name of Geliperm. This dressing can maintain the physiological wound environment during the first few critical days following grafting; it enables conditioning of the wound; it protects the wound against bacterial invasion and prevents drying-out of the graft and the base of the wound; and it is capable of absorbing wound secretion to a certain degree. Healing chances of the graft can be considerably improved by the use of Geliperm. Thanks to its high measure of elasticity it can adapt itself to the wound surface without sticking to it. The transparent nature of the material enables to observe and assess the healing processes at any time and to recognize possible complications during healing. Failures are almost always due to prolonged deposition of the gel plates, but this can be safely avoided by regularly changing the deposit. Deposits inadvertently left for too long, so that they have started drying at the wound surface, can be detached without any trouble and painlessly without any risk of damaging the graft.

Biomaterials. 1986 Jan;7(1):67-72.

Chemical and physical properties of a hydrogel wound dressing.

Kickhöfen B, Wokalek H, Scheel D, Ruh H.

Abstract

Geliperm hydrogel provides optimal physiological conditions for wound healing. The material is composed of two interlaced networks, one of polyacrylamide and one of agar, and contains about 96% firmly bound water. It is supplied in smooth, elastic, transparent sheets which are impermeable to bacteria but permeable to gases, salts, metabolites and proteins. Geliperm is nontoxic and has no irritative properties. Mechanical properties, water retention and diffusion of dyes and proteins are reported. Bacterial size should preclude penetration of the gel. The hydrogel in granular form represents a coherent material which could be used in deep fissured wounds and for the treatment of injuries with a large amount of exudation and contamination.

Fortschr Med. 1990 Feb 20;108(5):94-6.

Wound management with split skin flaps–donor sites. Covering with the moist gel Geliperm.

Sattler G1, Hagedorn M.

Abstract

In 23 patients, donor sites of split-thickness skin grafts were treated with Geliperm Hydrogel, a swellable polyacrylamide agar. Healing duration, toleration, exudation and pain were all noted during the daily change of dressing. In 22 of the 23 cases, good healing was obtained after an average of 12.3 days. We feel that Geliperm is excellently suitable for covering the donor sites of split-thickness skin grafts.

2/ PAAG HYDROGEL FOR TOPICAL DRUG DELIVERY SYSTEM

JAISWAL et al.

Polycaprolactone diacrylate crosslinked biodegradable semi-interpenetrating networks ***of polyacrylamide and gelatin for controlled drug delivery.***

Biomed Mater. 2010 Dec;5(6):065014

Biomed Mater. 2010 Dec;5(6):065014. doi: 10.1088/1748-6041/5/6/065014. Epub 2010 Nov 15.

Polycaprolactone diacrylate crosslinked biodegradable semi-interpenetrating networks of polyacrylamide and gelatin for controlled drug delivery.

Jaiswal M1, Dinda AK, Gupta A, Koul V.

Abstract

A biodegradable semi-interpenetrating hydrogel network (semi-IPN) of polyacrylamide and gelatin was prepared using polycaprolactone diacrylate (mol. wt ~ 640) as a crosslinker. The drug-polymer interaction and IPN formation were investigated by attenuated total reflectance-Fourier transform infrared (ATR-FTIR) and thermal gravimetric analysis (TGA). Scanning electron micrographs of lyophilized matrices revealed porous internal structure with varying pore sizes under equilibrium hydrated conditions, depending upon formulation composition. pH-dependent swelling and degradation was enhanced with increasing gelatin content and decreasing crosslinker concentration (Cs). Compression modulus (CM) (at 20% strain) increased significantly from 23 ± 1.4 to 75 ± 2.7 kPa ($p < 0.02$) with increasing Cs (from 0.5 to 2.0 mol%), while it decreased from 162 ± 6.4 to 23 ± 1.4 kPa ($p < 0.05$) with decreasing PAm/G ratio. Cell viability studies by MTT assay showed excellent cytocompatibility of matrices with fibroblast L929 cells. Curcumin, a hydrophobic phytochemical, was loaded by a diffusion method and its release profile was investigated in 4% w/v aqueous BSA solution at 75 rpm (at 37 ± 0.2 °C). Fitting of drug release data in the Korsmeyer-Peppas model suggested sustained release behavior up to 10 days with a combination of diffusion and erosion mechanism ($0.5 < n < 1.0$; $M(t)/M(\infty) \leq 0.6$). The newly developed porous, biodegradable and elastic semi-IPNs may serve as an ideal matrix for controlled drug release and wound healing applications. The possibilities can be explored for pharmaceutical and tissue engineering applications.

CHAPTER 2

TOPICAL OPHTALMOLOGY USE

EZRA et al.
Randomised trial comparing ocular lubricants and polyacrylamide hydrogel dressings
in the prevention of exposure keratopathy in the critically ill.
Intensive Care Med. 2009 Mar;35(3):455-61

EZRA et al.
Preventing exposure keratopathy in the critically ill: a prospective study comparing eye care regimes.
Br J Ophthalmol. 2005 Aug; 89(8): 1068–1069

EZRA et al.
Randomised trial comparing ocular lubricants and polyacrylamide hydrogel ***dressings in the prevention of exposure keratopathy in the critically ill.***
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EZRA et al.
Preventing exposure keratopathy in the critically ill: a prospective study comparing eye care regimes.
Br J Ophthalmol. 2005 Aug; 89(8): 1068–1069

Intensive Care Med. 2009 Mar;35(3):455-61. doi: 10.1007/s00134-008-1284-4. Epub 2008 Sep 23.

Randomised trial comparing ocular lubricants and polyacrylamide hydrogel dressings in the prevention of exposure keratopathy in the critically ill.

Ezra DG1, Chan MP, Solebo L, Malik AP, Crane E, Coombes A, Healy M.

Erratum in

Intensive Care Med. 2009 Mar;35(3):578.

Abstract

Purpose
To compare the cost and effectiveness of the two most popular forms of eye care in intensive care, ocular lubricant (Lacrilube) and polyacrylamide hydrogel dressings (Geliperm); for the prevention of exposure keratopathy in the critically ill.

Methods
A prospective randomised contralateral eye study was conducted at the Intensive Care Unit (ICU), Royal London Hospital, London, UK. Eighty eyes of 40 patients were recruited. Each patient received both Lacrilube and Geliperm allocated at random to different sides. A daily ophthalmology ward round was conducted. The outcome measures included the greatest palpebral aperture length, conjunctival oedema, and any exposure keratopathy.

Results
There was no statistically significant difference in the maximum corneal exposure score between the eyes treated with Lacrilube and Geliperm (P = 0.38). No significant difference in degree of chemosis or palpebral aperture was identified.

Conclusions
Our data suggest that Geliperm is as effective as Lacrilube in the prevention of exposure keratopathy in the critically ill. We also note that nursing staff must be fully trained in its application for eye care.

Br J Ophthalmol. 2005 Aug; 89(8): 1068–1069. doi: 10.1136/bjo.2004.062406

Preventing exposure keratopathy in the critically ill: a prospective study comparing eye care regimes.

D G Ezra, G Lewis, M Healy, and A Coombes

Microbial keratitis has been reported among critically ill patients and the need for effective eye care in the intensive care unit (ICU) has been recognised for some time.1 However, different eye care regimes are not always evidence based2 and there is no clear consensus defining the best form of eye care. A recent survey in the United Kingdom found that 75% of ICUs used Geliperm routinely as eye care, with 25% using ocular lubricants3 Although Geliperm was originally designed as a wound dressing and there is no evidence to support its use in eye protection. Lacrilube, however, has been shown to be effective in reducing exposure keratopathy in sedated and paralysed patients.4 This prospective comparative study aims to assess the prevalence of corneal surface disease in ICU and the effectiveness of two different eye care regimes at preventing corneal surface disease.

Methods

Three main types of eye care are instituted at the discretion of nursing staff: (1) simple eye toilet; (2) Lacrilube alone; (3) Geliperm alone.

Patients admitted over a 4 month period were examined at weekly ophthalmology ward rounds for signs of ocular surface disease. All patients who spent less than 3 days on the unit and with primary orbital injury were excluded. The type of eye care regime was recorded as well as greatest vertical diameter of the palpebral aperture (mm), conjunctival chemosis, and length of stay. The cornea was assessed by instillation of fluorescein and viewing with cobalt blue light using an indirect ophthalmoscope and 20 dioptre lens. Corneal damage was graded from 1–6

according to severity using a previously described grading system (table 11).). Conjunctival chemosis was graded from 1–3 (table 22)) The sedation score and number of days that the patient were in the ICU were also recorded. Any patient found to have a compromised cornea was removed from the study and treated with prophylactic antibiotic ointment.

Results

Forty seven patients were recruited. A total of 24 were found to have exposure keratopathy (50%). These results are summarised in table 33.. Twenty four patients were identified who received basic eye toilet alone (no Lacrilube or geliperm). Of these, 13 patients (54%) were found to have exposure keratopathy. Thirteen patients received Lacrilube as prophylaxis and two (15%) of these patients developed exposure keratopathy. Ten patients were treated with Geliperm alone and of these nine (90%) were found to have exposure keratopathy. In general, more severe keratopathy was seen in the Geliperm group. Statistical comparison of the three groups indicated that Lacrilube is a better prophylactic measure at preventing keratopathy than basic eye care alone (Fisher's exact test p=0.04), and more effective than Geliperm (Fisher's exact test p=0.001).

No significant variance was detected in the groups between sedation score (p=0.45 Kruskal-Wallis) and number of days in the ICU (p=0.09 Kruskal-Wallis). No skew in other ophthalmic variables, such as degree of conjunctival chemosis (Kruskall-Wallis p=0.056) and palpebral aperture was found, with no significant variance between the groups (Kruskal-Wallis p=0.41)

Comment

Microbial keratitis is almost always preceded by compromise of the corneal epithelium. The immune defences of the eye are predominantly innate and consist of a combination of mechanical, anatomical, physiological, and barrier defence mechanisms. These include an intact corneal epithithelium and the constant blinking action of the eyelids. The tear film also has important antimicrobial components such as lactoferrin, β lysin and immunoglobulins.5

ICU patients are often sedated and paralysed leading to incomplete eyelid closure. Critical illness is frequently associated with capillary leak and fluid retention that causes peripheral oedema and conjunctival oedema. As a result, these patients are susceptible to exposure keratopathy.6 The breakdown of the innate ocular defences of the eye is known to predispose to opportunistic infection. There have been many reports of Pseudomonas and Acinetobacter infections causing microbial keratitis among the critically ill.7 It is thought that procedures such as endotracheal suctioning may lead to aerosol inoculation of the susceptible patients' corneal surface by respiratory tract organisms.8,9 The need for effective eye care has been recognised for some time10 and although data exist to compare moist chamber treatments with ocular lubricants,11,12 no data exist to compare the efficacy of polyacrylamide Hydrogelt (Geliperm) and ocular lubricants. We also note that Geliperm has been designed as a wound dressing and does not have a devices licence for eye care.

Our data suggest that the use of Lacrilube is more effective than Geliperm or basic eye care. Further research is clearly needed in this area.

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CHAPTER 3

OVERVIEW OF VARIOUS MEDICAL USE OF (PAAG) HYDROGELS

ENRICA CALÓ et al.
Biomedical applications of hydrogels: A review of patents and commercial products.
European Polymer Journal 65 (2015) 252–267

LEE et al.
Voice outcomes of polyacrylamide hydrogel injection laryngoplasty.
Laryngoscope 117: October 2007

ENRICA CALÓ et al.

Biomedical applications of hydrogels: A review of patents and commercial products.

European Polymer Journal 65 (2015) 252–267

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Review Article

Biomedical applications of hydrogels: A review of patents and commercial products

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ABSTRACT

Hydrogels have become very popular due to their unique properties such as high water content, softness, flexibility and biocompatibility. Natural and synthetic hydrophilic polymers can be physically or chemically cross-linked in order to produce hydrogels. Their resemblance to living tissue opens up many opportunities for applications in biomedical areas. Currently, hydrogels are used for manufacturing contact lenses, hygiene products, tissue engineering scaffolds, drug delivery systems and wound dressings. This review provides an analysis of their main characteristics and biomedical applications. From Wichterle's pioneering work to the most recent hydrogel-based inventions and products on the market, it provides the reader with a detailed introduction to the topic and perspective on further potential developments.

Contents

1. Introduction

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of absorbing large amounts of water or biological fluids. Due to their high water content, porosity and soft consistency, they closely simulate natural living tissue, more so than any other class of synthetic biomaterials. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve [1].

Hydrogels are called 'reversible' or 'physical' gels if molecular entanglements and/or secondary forces such as ionic, H-bonding or hydrophobic forces play the main role in forming the network. Physical gels are often reversible

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0014-3057/

and it is possible to dissolve them by changing environmental conditions, such as pH, and the ionic strength of solution or temperature. In 'permanent' or 'chemical' gels, the network of covalent bonds joining different macromolecular chains can be achieved by cross-linking polymers in the dry state or in solution [2]. These gels may be charged or non-charged depending on the nature of functional groups present in their structure. The charged hydrogels usually exhibit changes in swelling upon variations in pH, and it is known that they can undergo changes in shape when exposed to an electric field [3].

Chemical hydrogels are commonly prepared in two different ways: 'three-dimensional polymerization' (Fig. 1), in which a hydrophilic monomer is polymerized in the presence of a polyfunctional cross-linking agent, or by direct cross-linking of water-soluble polymers (Fig. 2). Polymerization is usually initiated by free-radical generating compounds such as benzoyl peroxide, 2,2-azo-isobutyronitrile (AIBN), and ammonium peroxodisulphate or by using UV-, gamma- or electron beam-radiation. However, three-dimensional polymerization often results in materials containing significant levels of residual monomers and therefore purification of these materials has to be performed thoroughly because the unreacted monomers are often toxic and could leach out from the hydrogels continuously. The purification of hydrogels containing residual monomers is typically performed by extraction into excess water, and can take up to several weeks to be completed [4–7].

There are numerous approaches that could be used to improve or avoid the purification process. One possibility is the use of additional processes that lead to the highest possible degrees of monomer conversion, which could be achieved by conducting three-dimensional polymerization followed by subsequent post-polymerization curing (e.g. by thermal treatment or irradiation of the resulting products) [8,9]. Alternatively, the selection of non-toxic monomers used for the three-dimensional polymerization, such as oligomers or macromonomers (e.g. polyethylene glycol dimethacrylate) could be a solution [10].

It is also possible to avoid the need for purification of hydrogels after their synthesis by cross-linking ready-made water-soluble polymers. Water-soluble polymers such as poly(acrylic acid), poly(vinyl alcohol), poly(vinylpyrrolidone), poly(ethylene glycol), polyacrylamide and some polysaccharides are the most common systems used to form hydrogels. These water-soluble polymers are non-toxic and widely used in various pharmaceutical and biomedical applications and therefore do not require removal from the system, eliminating the need for a purification step. Radiation induced cross-linking, such as of an aqueous solution of hydrophilic polymers with gamma rays, allows simultaneous formation of a hydrogel and its sterilization. Rosiak et al. [11,12] used cross-linking of natural polymers (such as gelatine or agar) and synthetic polymers (such as poly(vinyl pyrrolidone) (PVP) or poly(vinyl alcohol) (PVA) which were cross-linked by gamma radiation for the production of sterile hydrogels used in wound care. Currently their hydrogels are manufactured and marketed as 'Kikgel' and 'Aqua-gel' wound dressings [11,12].

Recently, Khutoryanskiy et al. [4,13] reported an alternative method to synthesise hydrogels from ready-made water-soluble polymers in aqueous solutions using thermal treatment or microwave irradiation. In this method

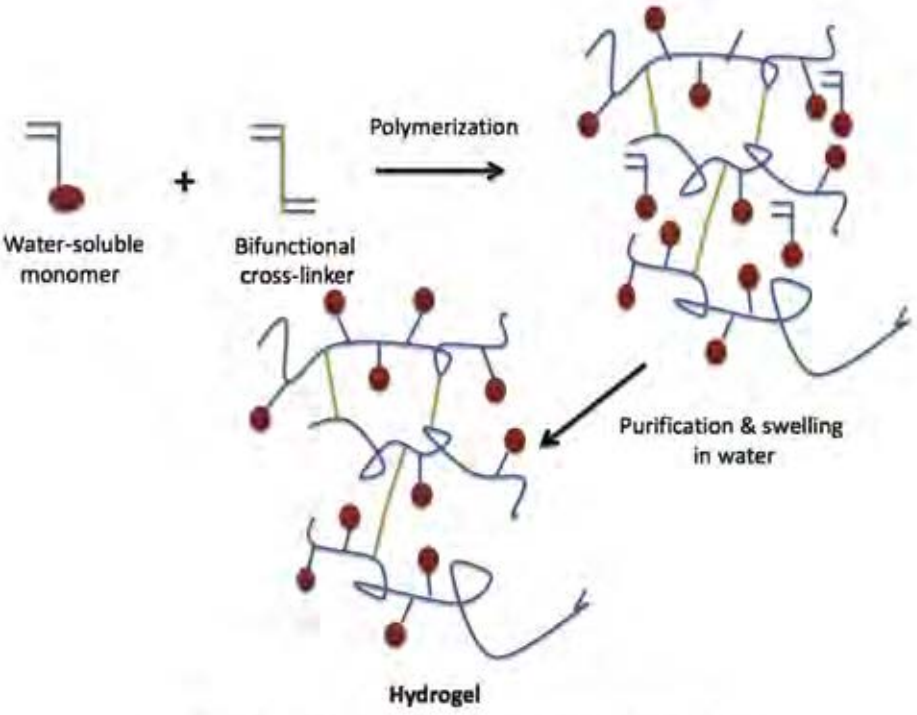


Fig. 1. Synthesis of hydrogels by three-dimensional polymerization.

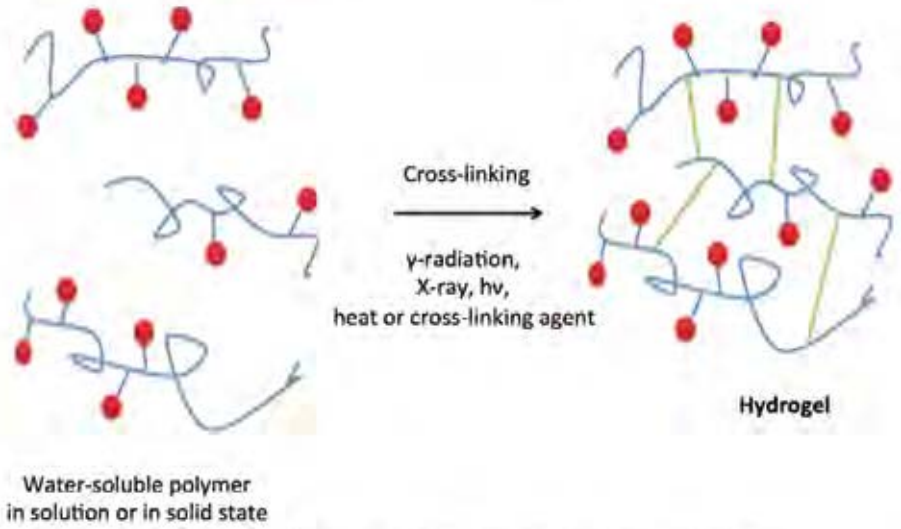


Fig. 2. Synthesis of hydrogels by cross-linking of ready-made water-soluble polymers.

the aqueous solutions of specific water-soluble polymers such as poly(methyl vinyl ether-*alt*-maleic anhydride) and poly(vinyl alcohol) are mixed together at room temperature and the cross-linking process is achieved by thermal treatment under high pressure via autoclaving or microwave radiation. Both radiation and thermal cross-linking methods are inexpensive, safe, do not require a purification step and result in sterile hydrogels if a suitable combination of hydrophilic polymers is used.

There are numerous original papers, academic reviews and monographs focused on the synthesis, properties and applications of hydrogels [1,3,14–22]. This review will consider mostly patent literature on 'chemical' hydrogels and their potential commercial applications in biomedical areas. As shown by the considerable number of patents and commercial products, the main areas of hydrogel applications are contact lenses, wound dressings, drug delivery systems, tissue engineering, and hygiene products; these will be covered in this review.

2. Contact lenses

In their pioneering 1960 paper, Wichterle and Lim were the first to describe a hydrogel based on poly-2-hydroxyethylmethacrylate (PHEMA) as a synthetic biocompatible material useful for contact lens applications [23]. PHEMA lenses were distributed firstly in western Europe in 1962, but with limited success. In 1965 the National Patent Development Corporation (NPDC) bought the licence to this technology. This was then sold to Bausch & Lomb, which optimised Wichterle's spin-casting process and finally acquired the approval from the Food and Drug Administration (FDA) for their PHEMA lenses in 1971 [24].

Contact lenses are mainly classified as 'hard' or 'soft' according to their elasticity. Even though hard lenses are longer lasting, they tend to be poorly accepted by the wearers and can require a lengthier adaptation period. Hard contact lenses are primarily based on hydrophobic materials such as poly(methyl methacrylate) (PMMA) or poly(hexa-fluoroisopropyl methacrylate) (HFIM), whereas soft lenses are based on hydrogels [25].

Soft contact lenses can be produced with different techniques, such as spin-casting, mold-casting and lathe-cutting. In spin- and mold-casting a small amount of liquid monomer mixture is placed into special 'concave' optical molds in order to shape the lens. During spin-casting the concave mold rotates to form the lens, causing the liquid monomer to flow out uniformly, coating the whole surface. At the same time, polymerization of the monomer is carried out at elevated temperatures, and the residual monomer is carefully removed at the end of the process. The mold-casting technique employs a convex mold which is inserted into the liquid monomer which already contains a mated concave mold, to make the back surface of the lens. The polymerization takes place in the same way as for the spin-casting. This process produces hard lens interposed between the optical surfaces of the two different molds and once the lens is dry it remains concave [26]. Innovative molds, useful for cast molding silicone hydrogel contact lenses, have been described in the US Patent 6,861,123 B2 assigned to Johnson & Johnson Vision Care Inc. Turner et al. [27] patented their polyolefin inserts for producing the molds and the method in which these are used to make lenses. The preferred method for producing the aforementioned lenses was by direct molding of the silicone hydrogels, placing the reaction mixture in a mold having the shape of the final desired product, and then proceeding with the polymerization.

An alternative method used in the contact lens industry is lathe-cutting (Fig. 3), in which the lenses are formed from solid 'buttons' of dehydrated material. The liquid monomer mixtures are usually bulk-polymerized in water tanks for some period of time. This type of polymerization is typically started using free-radical initiators which are then decomposed by an increase in temperature. This process results in the formation of longer polymer chains (with higher molecular weights) and potentially more chain entanglements. Oxygen-mediated degradation could

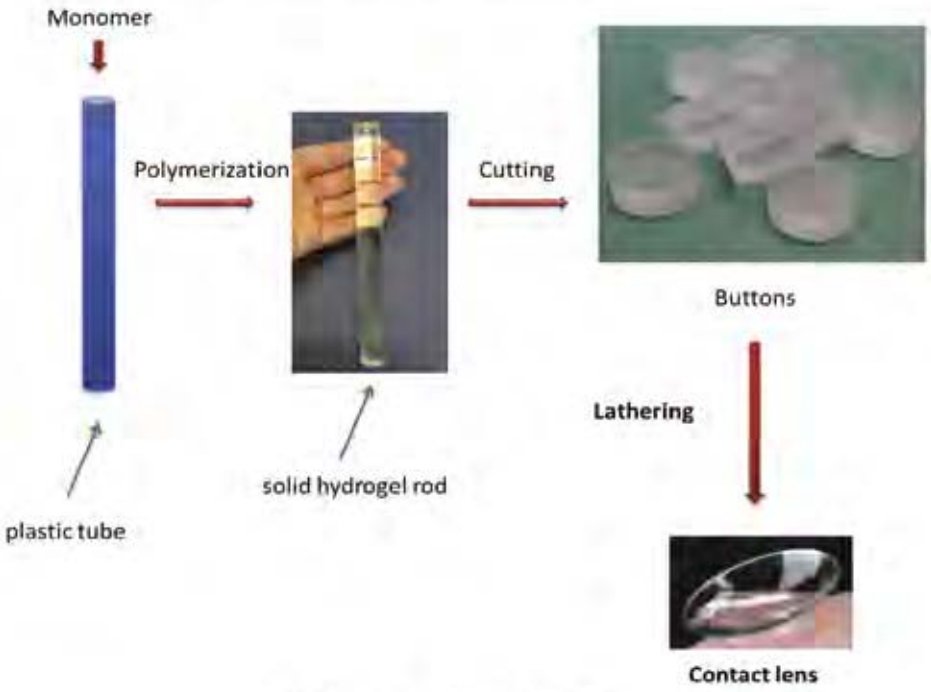


Fig. 3. Scheme of lathe-cutting technique.

Table 1
Requirements to the hydrogels used for contact lens applications.

Characteristics	Requirements	References
Luminous transmittance	The minimum luminous transmittance value for contact lenses is 95%. This value significantly affects the transparency of the lens. A slit lamp microscope is typically used to observe any deposit (proteins, lipids, bacteria, minerals) that may cause a lack of the usual transparency of the lens	[28,29]
Refractive index	The refractive index of the human cornea surface may vary. Ideal hydrogels should have a refractive index value matching the range 1.372–1.381	[40,41]
Sufficient oxygen-permeability	The oxygen permeability of the lens is directly proportional to water content and inversely to thickness. In the contact lens industry the oxygen permeability is expressed as *Dk*. In order to prevent anoxia throughout the cornea the oxygen transmissibility of the lens (*Dk/t*, where '*t*' is the thickness of the lens) required to be 35 for the open eye and 125 for the closed eye	[33–34]
Wettability and permeability to water	The water-permeability of the lens is strictly related to thickness. A constant water diffusion rate is normally reached within the first hour of the experimental analysis. It directly depends on the wettability of the lens, which is evaluated by advancing contact angle ($\Theta w/a$) measurements. The initial value for hydrogel contact lens is usually around 25°	[35,36]
Stability	The stability of the material used affects the shelf-life and the manufacturing process of the lens	[37]
Excellent mechanical properties	The mechanical properties of the lens, such as the elastic modulus (E), have a great impact on their adhesion to the corneal epithelium and on the comfort for the wearer	[38]
biocompatibility	The biocompatibility of the material is essential for the ocular health of wearers who tend to use the lenses for an extended period of time	[39]

occur at the surfaces but a button will have a moderately high volume to surface ratio, so it is possible to remove the surfaces during the lathing process. The lenses are finally collected from the centre of a button [24].

A polymeric hydrogel should have some important physical properties to be used as a contact lens material [24]. The ideal characteristics are listed in Table 1:

The equilibrium water content (EWC) of a hydrogel is defined as:

$$EWC = m/m_{tot} \times 100\% \quad (1)$$

where m is the weight of water in polymer and m_{tot} is the total weight of hydrated polymer.

EWC could change with temperature, pH and osmolality. For example, PHEMA contact lenses contain approximately 38–40% of water in the fully hydrated state. They typically show low variability with changes in external factors [24].

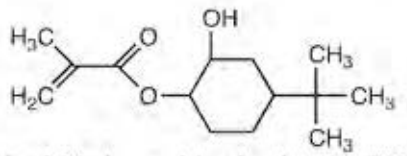
In US Patent 3,679,504 [40], Wichterle disclosed a method of forming colored soft contact lenses and ophthalmic prostheses. The colored ingredient was incorporated between two transparent hydrogel layers bound together by polymerizing the hydrophilic monomers mixture. The covering hydrogel layer could also be made from a solution of a hydrophilic macromonomer such as polyethylene glycol mono-methacrylate, which could be manufactured as

described in US Patent 3,575,946 [41]. The use of macromonomers for preparation of hydrogels can potentially eliminate the need for their purification as these materials are often non-toxic [40].

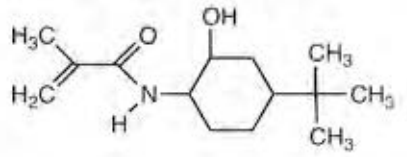
In US Patent 4,472,327 [42] Neefe proposed a method of making cosmetic hydrogel contact lenses which modified the apparent color of the iris by using small light reflecting particles imbedded in a colored transparent matrix. The lenses described in this patent are of a dual purpose: to correct the visual defects and to change the apparent color of the eye. The whole lens area was transparent, providing peripheral vision and allowing the natural iris pattern to be visible through them. Neefe discovered that when a small amount of high refractive index fine particles was placed in a matrix of transparent lens material of a substantially lower refractive index, the reflected light had the color of the lower refractive index media [42]. Selected particulate material had been employed in the polymerization of HEMA with benzoyl peroxide as an initiator. Furthermore, it was possible to add a selected antimicrobial agent, for example 3-(trimethoxysilyl)propyloctadecylmethyl ammonium chloride, to the liquid monomer mixture before polymerization to ensure that the resulting lenses were more resistant to microbial growth [24].

Numerous attempts have been made to develop new contact lenses with better physical and chemical properties. For instance, Jay Kunzer and Friends [43] disclosed that certain hydrophobic monomers, such as those shown in Fig. 4, can act as strengthening agents when copolymerized with hydrophilic monomers such as HEMA, or N-vinyl-2-pyrrolidone (NVP).

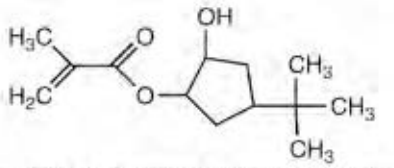
The soft contact lenses made from these monomers combined with HEMA or NVP are large enough to cover the whole cornea and present good oxygen permeability, ensuring more comfort for wearers [43].



4-t-butyl, 2-hydroxycyclohexyl methacrylate (TBE)



methacryloylamino-4-t-butyl 2-hydroxycyclohexane



4-t-butyl, 2-hydroxycyclopentyl methacrylate

Fig. 4. Some of the hydrophobic monomers used by Kunzer et al. [43].

Lai and Quinn [44] proposed the use of a new class of optically clear silicone thermoplastic hydrogel materials in the production of contact lenses. The general formula of the polymers, which includes a silicone-containing segment derived from polysiloxane linked with hydroxyl or amino groups, is shown in Fig. 5.

These materials offered good physical strength and excellent oxygen permeability. A drawback of 'soft' contact lenses in general is their relatively poor gas permeability resulting in oxygen deprivation of the cornea, which receives oxygen only from the atmosphere. In the contact lens industry oxygen permeability is defined as '*Dk*', where '*D*' is the diffusivity of the lens and '*k*' is the oxygen solubility in the lens material [24]. *Dk* essentially depends on EWC in conventional hydrogels because oxygen has the capability to diffuse through water rather than through the gel. The relationship between these two parameters is:

$$Dk = 1.67e^{(0.0397EWC} \quad (2)$$

where '*e*' is the base of the natural logarithm. Oxygen transmissibility of contact lenses may be calculated from the *Dk* of the material divided by the lens thickness (*t*). The units of *Dk* are called Fatt units (named after Professor Irving Fatt) or Barrer [24]:

$$Dk(\text{barrer}) = 10^{-11}(\text{cm}^2 \times \text{mLO}_2)/\text{sec} \times \text{mL} \times \text{mmHg} \quad (3)$$

$$Dk/t \quad Dk(\text{barrer/cm}) = 10^{-9}(\text{cm} \times \text{mLO}_2)/\text{sec} \times \text{mL} \times \text{mmHg} \quad (4)$$

Nowadays, silicone hydrogel (SiHy) lenses have become prevalent on the market (Fig. 6), due to their higher oxygen permeability and comfortable fit [45].

One of the drawbacks associated with the use of SiHy lenses is that they often undergo more protein deposition than conventional lenses which leads to problems with lens spoilage. In European Patent EP 2 365 360 A2, a method for reducing protein deposition on contact lenses has been proposed by adding protein uptake-reducing compounds, such as butylated hydroxytoluene (BHT) or hydroxyamines in the reaction mixture [46]. The reaction mixture may include a 'silicone-containing monomer', described in the US Patent 3,808,178 (Fig. 7) [47].

Contact lens surfaces should also have excellent wettability in order to avoid tear-film deposits [47]. The SiHy lenses have been made to compensate the hydrophobicity of silicone and to improve its wettability. The silicone hydrogel lenses were molded and plasma-treated afterwards [49]. The clinical performance of any contact lens material depends on its ability to produce a stable pre- and post-lens tear film, which is dependent on its wettability. This can be described as the formation of a continuous superficial fluid film over a solid surface. The wettability index is usually determined by measuring the contact angle (α) of water on a lens surface. If $\alpha = 0°$ then water is able to fully wet the lens, if $\alpha < 90°$ water wets the lens and if $\alpha > 90°$ the lens is practically not wettable [48].

'Soft' lenses with greater adherence to the eye have been developed in order to enhance the fit, but on the other hand, they have poor gas permeability and often do not allow oxygen to reach the cornea at a sufficient rate.

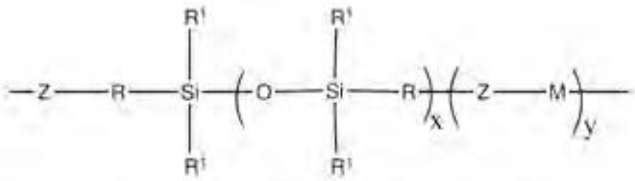


Fig. 5. General formula of the thermoplastic silicone-containing compositions employed by Lai et al. 'M' is a hydrophilic group; 'R' is an alkyl group with 1–10 carbon atoms that can be separated by ether, urethane or ureido linkages; 'R^1' is hydrogen, monovalent hydrocarbon groups or halogen substituted monovalent hydrocarbon moieties with 1–18 carbon atoms; 'Z' may be a divalent urethane or ureido portion; 'x' and 'y' are equal or greater than 1 [44].

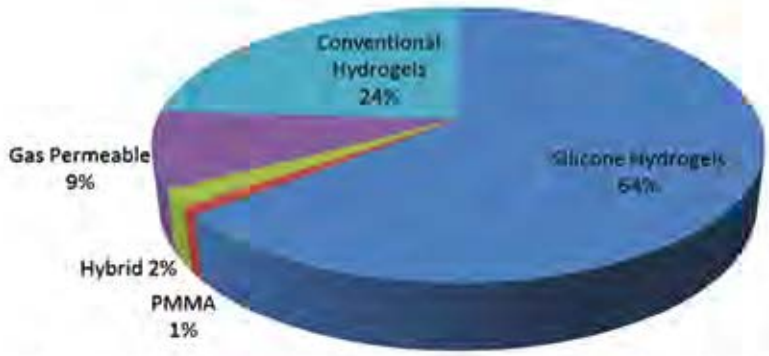


Fig. 6. Materials used in contact lenses manufacturing in 2012. Data for this figure was taken from [45]. Reprinted with permission from Contact Lens Spectrum, published in January 2013. Contact Lens Spectrum is published monthly by PentaVision LLC© 2014 All Rights Reserved. PentaVision is located at 321 Norristown Road, Suite 150, Ambler, PA 19002 (USA). Please visit www.contactlensspectrum.com for more information.

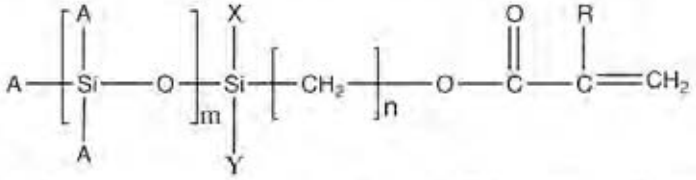


Fig. 7. General formula of polysiloxanylalkyl ester monomer presented in US Patent 3,808,178. A, X and Y can be C_1–C_5 alkyl groups or phenyl groups; R is a methyl group or hydrogen; m is an integer from 1 to 5 and n is from 1 to 3 [47].

SiHy contact lenses have been designed to overcome this problem, because they are composed of hydrated, cross-linked polymeric material that contains silicon and a certain amount of water within the polymer matrix. More recently, Bauman et al. [50] disclosed a method for making SiHy contact lenses with a nano-textured surface, imitating the surface of human cornea. The nano-textured surface coating technique has been developed through controlled soaking of the lens into a polymeric material, which can comprise monomeric units of one or more carboxyl-containing vinylic monomers. The nano-textures are then fixed by crosslinking a water-soluble hydrophilic polymeric material onto the prime coating.

One of the more recent products of the UK contact lens industry is 'Gentle 59', promoted by Vista Optics Ltd. at the European Federation of the Contact Lens and Iols Industries Congress in Budapest in September 2012. Gentle 59 is made of an acrylic acid-*co*-acrylamide hydrogel, which seems to have very good tensile properties, moisture retention characteristics and a very comfortable fit [51].

In addition to the applications of soft contact lenses in correction of vision, they can potentially be used for drug delivery to the eye. However, conventional hydrogel-based contact lenses exhibit relatively low drug loading capacity and often show a burst release upon ocular administration [52]. Many methods have been developed to modify the conventional contact lenses to improve their drug loading and release. These include modifying the polymeric materials with a controlled hydrophilic/hydrophobic copolymer ratio, impregnating drug-containing colloidal structures, incorporating ligand-including hydrogels and developing multilayered hydrogels [52]. Venkatesh et al. [53] showed the potential of 'biomimetic hydrogels' as carriers to load relevant amounts of H1-antihistamines. They also show potential to release therapeutic dosages of drug in vitro in a controlled manner for a period of 5 days, with a possible extension in the presence of proteins. Xu et al. [54] incorporated β-cyclodextrin (β-CD) into hydrogels for contact lenses, observing an increase in the equilibrium swelling ratio and tensile strength. Puerarin was used as a

model drug to study loading and release from PHEMA/beta-CD hydrogels. It was established that puerarin loading and the in vitro release rate depended on the amount of beta-CD in the hydrogel. In rabbit eyes the PHEMA/beta-CD hydrogel contact lenses demonstrated longer residence time of puerarin in the tear fluid compared to conventional PHEMA contact lenses and 1% puerarin eye drops.

Developing safe and cost-effective contact lenses is the focus of the eye care industry. Contact lens materials with optimal characteristics such as oxygen permeability, comfort, compliance, hygiene and disinfection have still not been achieved, which opens exciting opportunities for further developments in this area.

3. Wound dressings

A wound is a defect or a break in the skin which can result from trauma or medical/physiological conditions. Wounds can be classified, depending on the number of skin layers and on the area of the skin affected, as superficial (if only the epidermis is involved), partial-thickness (if the epidermis and deeper dermal layers are affected) and full-thickness wounds (when subcutaneous fat and deeper tissue has been damaged) [55]. Wounds are usually subdivided into 'acute' or 'chronic' wounds. Chronic wounds require dedicated nursing care that represents a significant cost for national health systems. Design of effective dressings relies on an understanding of the healing process, as well as the specific conditions of a patient and the effect that each material used could have on the wound [55,56]. Wound healing can be hindered by various factors such as desiccation, infection or abnormal bacterial presence, maceration, necrosis, pressure, trauma and edema [57].

Table 2
Advanced wound dressings (reprinted from P.S. Murphy, G.R.D. Evans, Plastic Surgery International 2012, 2012, 1) [60].

	Notes
Protective dressings	
Gauze	Inexpensive; readily available
Impregnated gauze	Nonadherent; preserves moisture
Antimicrobial dressings	
Antibacterial ointments	Reapply often to maintain moisture
Iodine based	Absorbent; Not for use with thyroid disorders
Silver based	Many forms; Broad spectrum; low resistance
Autolytic debridement	
Films	Occlusive; allows exchange of gasses
Hydrocolloids	Not for exudative or infected wounds
Hydrogels	Rehydrates to soften dry wounds
Chemical debridement	
Papain/urea	Availability issues in US
Collagenase	Selective debridement
Absorbent dressings	
Foam	Absorbs moderate exudate
Hydrogels	Absorbs minimal exudate
Hydrofibers	Absorbs heavy exudate
Alginates	Absorbs heavy exudate

The 'ideal' wound management product should absorb excess exudate and toxins, keep a good moisture between the wound and the dressing, preserve the wound from external sources of infection, prevent excess heat at the wound, have good permeability to gases, be supplied completely sterile and be easy to remove without further trauma to the wound [58].

Recently, the wound dressing industry highlighted the importance of providing comfort and conformability of dressings, the need for infrequent changes, cost effectiveness and a long shelf life [58]. The choice of the right dressing to suit a particular wound is therefore fundamental for optimum healing and the quality of life of the patient [59]. The majority of the currently available products can be classified as low adherent dressings, semipermeable films, hydrocolloids, hydrogels, alginates, foam dressings or antimicrobial dressings [57]. Although plain gauze is still one of the most commonly employed products in hospitals, new wound dressing research and development has produced advanced materials with better physical and chemical properties (Table 2). Gauze is certainly cheap, readily available and suitable for a lot of wounds. In particular the gauzes impregnated with some active ingredients such as iodine, zinc oxide/zinc ions, or petrolatum show enhanced performance. Iodine provides antimicrobial properties, whereas zinc oxide could promote wound cleansing and re-epithelialization [60,61]. However, the use of gauze often results in problems associated with its removal as it may cause trauma by stripping off newly formed epidermis [62].

Advanced dressings are designed to maintain a moist environment at the site of application, allowing the fluids to remain close to the wound but not spread to unaffected, healthy skin areas [62]. The relevance of the moist wound environment as a factor accelerating the healing process was first observed by Winter in 1962, but only recently has received more serious attention [63]. Dressings designed for moist wound healing are represented by hydrogel and hydrocolloid products but only the latter can absorb mild to medium exudate or drainage. Both induce autolytic debridement, which facilitates the elimination of the dead tissue [57]. Hydrocolloids are usually composed of sodium carboxymethylcellulose, gelatin, pectin, elastomers and adhesives. Hydrofiber®(ConvaTec) dressings allow moisture to be captured because they form a swollen gel structure and conform to the wound site forming a 'seal'. Hydrofiber® may be in the form of a hydrophilic, non-woven flat sheet dressing that can be converted to a soft gel sheet by absorbing the wound exudate [58].

Hydrogels are widely used as debriding agents, moist dressings, and components of pastes for wound care. However, they do not need further wound fluids to become gels and are suitable for dry wounds [60].

The so-called 'moisture donor' effect of hydrogels helps autolytic debridement, increasing collagenase production and the moisture content of necrotic wounds [62]. They can absorb and retain contaminated exudate within the gel mass through expansion of crosslinked polymer chains resulting in isolation of bacteria, detritus and odour molecules in the liquid. Their high water content allows vapor and oxygen transmission to the wounds such as pressure

sores, leg ulcers, surgical and necrotic wounds, lacerations and burns. They seem to play an important role as emergency burns treatment alone or in combination with other products, thanks to their cooling and hydrating effect [63]. For example, Burnshield hydrogel burn dressing (Levtrade International) present even in first aid kits is a polyurethane foam containing 96% of water and 1.06% *Melaleuca alternifolia* extract [64].

Hydrogel dressings are also used for granulating cavity wounds [65]. Amorphous gels are generally reapplied every day while sheet hydrogels are usually changed 2–3 times a week [66] (see Table 3).

In 1992 Cartmell and Sturtevant [72] proposed a transparent wound dressing as thin-film, with a non-adhesive central portion containing hydrogel material which included polypropylene glycol or polyethylene glycol, and isophorone diisocyanate. This product is described as being flexible in order to facilitate its removal, and transparent to permit constant observation of the wound healing process. Cartmell describes that the edges of this dressing adhere to the skin due to the adhesive layers that protect the wound site from bacteria and foreign bodies. Two years later in the US Patent 5,423,737 Cartmell at al. [73] disclosed an improved version of this transparent wound dressing. In this case there was a release tab inserted between the transparent layer and the release liner. The invention was intended to respond to a need for a cost-effective product which was simple to manufacture and easy to handle and apply. A similar device has been presented by Holm et al. [74], in which a hydrogel pad is included within an adhesive dressing. This demonstrates that many attempts have been made using new technologies but having the same patient goals.

If local or systemic infection is compromising the wound, or could compromise the healing process, one possible therapeutic approach would be to use dressings containing antimicrobial agents, such as iodine or silver. Silver is useful against a large range of microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* [75]. These two opportunistic pathogens are frequently present in chronic wounds and their mechanism of action includes a biofilm-based infection in the host [76]. A 'critical colonisation' resulting from a multiplication of bacteria is normally accompanied by an increase in pain. Even if the correct treatment is chosen, the healing process could be delayed by a 'critical colonisation' which can result in the formation of a thick slough that is not responsive to standard debridement techniques and a malodour. Bacteria levels should be reduced to a minimum to allow the wound to heal, and the topical application of an antimicrobial dressing is one of the most common ways to achieve this effect [75]. US Patent 8,431,151 B2 proposed a method to manufacture a hydrogel antimicrobial non-woven fibrous dressing with controlled release of silver ions. The inventors describe a PEG-based multi-block thermoplastic polyurethane incorporating polyhedral oligomeric silsesquioxane, forming organic–inorganic hybrid hydrogels with unique mechanical properties and adjustable swelling ratios. In this case a nanofiber network, produced with the electro-spinning technique, was used to deliver silver ions. $AgNO_3$ was directly incorporated into polymer/dimethylformamide solutions to prepare the antimicrobial scaffolds [77].

Hydrogels have been included in the structure of some wound dressings together with other materials, forming composite products suitable for many types of wounds. Shah et al. [78] described a material composed of a cotton gauze, or other fibrous substrate, impregnated with a thermoplastic hydrogel forming polymer. The polymers included A–B–A block copolymers, multiblock copolymers, graft copolymers and polymer blends each incorporating a hydrophilic (such as polyethylene oxide or poly(hydroxyalkyl methacrylate)) and a hydrophobic component (such as polystyrene, poly(methyl methacrylate) or polyesters). The hydrogel showed microphase separation of the hydrophobic portion becoming water-insoluble but remaining water-swellable. By absorbing the wound exudate, the composite dressing could assume a slimy consistency avoiding the adherence to the wound surface that could cause further trauma, and allowing more infrequent changes.

Future developments in wound care products will depend on continued demands from public and healthcare professionals [79]. The important challenge for the future is to establish the appropriate wound care strategy for every single patient, and this can be achieved only by offering the optimal products. Innovative dressings need to be developed while their production costs must be kept low.

4. Drug delivery

Many patents and academic papers about possible applications of hydrogels in drug delivery have been

Table 3
Some examples of hydrogels and hydrogel sheets as wound dressings.

Product	Main constituents	Main characteristics
Granugel® (ConvaTec)	Pectin, carboxymethylcellulose and propylene glycol	A clear, viscous hydrogel for the management of partial and full-thickness wounds, may be used as a filler for dry cavity wounds to provide a moist healing environment [67]
Intrasite Gel® (Smith & Nephew)	Modified carboxymethylcellulose (2.3%) and propylene glycol (20%)	Amorphous sterile hydrogel dressing for use in shallow and deep open wounds [68]
Purilon Gel® (Coloplast)	Sodium carboxymethylcellulose and more than 90% of water	Indicated in conjunction with a secondary dressing for necrotic and sloughy wounds and first and second degree burns [69]
Aquaflo™ (Covidien)	Polyethylene glycol and propylene glycol	It has a disc shape that maximizes wound coverage and helps to fill shallow cavities. Translucent gel that allows wound visualization [70]
Woundtab® (First Water)	Sulphonated copolymer, carboxymethylcellulose, glycerol and water	The dressing contains a superabsorbent polymeric gel able to absorb bacteria and retain them in its structure. Described as a wound 'kick-starter' patch for chronic wounds, it can also be used as a secondary absorbent [71]

published, however, only a few have resulted in commercial products. Hydrogels have attracted noticeable interest for their use in drug delivery due to their unique physical properties [80–82]. The high porosity that characterizes hydrogels can easily be adjusted by controlling the density of cross-links in their matrix and the affinity to water. Their porous structure also allows drugs to be loaded and then released. The advantages offered by hydrogels for drug delivery applications include the possibility for sustained release, which results in maintaining a high local concentration of an active pharmaceutical ingredient over a long period [80]. The drug can be loaded into a hydrogel and then its release may proceed through several mechanisms: diffusion controlled, swelling controlled, chemically controlled and environmentally-responsive release.

The diffusion controlled release systems can be represented by reservoir or matrix devices. Both allow the drug release by diffusion through the hydrogel mesh or the pores filled with water. A reservoir delivery system (Fig. 8) includes a drug-containing core coated with a hydrogel membrane, commonly available as capsules, cylinders, spheres or slabs. The concentration of the drug is higher in the centre of the system to allow a constant release rate [83].

In matrix systems the drug is dispersed or dissolved uniformly throughout the three-dimensional structure of the hydrogel (Fig. 9). Drug release is achieved through the macromolecular mesh or the pores, and the initial release rate in this case is proportional to the square root of time, rather than being constant and time independent as happens in reservoir systems [83].

In swelling-controlled release devices the drug is dispersed within a glassy polymer as in a matrix device, and when the polymer is in contact with a bio-fluid it starts swelling. The material then expands beyond its boundary allowing the diffusion of drug with the relaxation of polymer chains [83]. This process is also called Case II transport and it shows constant, time-independent kinetics of release. It is known as 'anomalous transport', one that combines swelling-controlled release with diffusion [84]. The gradient existing between the dispersed drug in the hydrogel and the surrounding environment permits the diffusion of the active ingredient loaded from the high concentration through the hydrogel, to the lower one [85]. The molar flux of the drug in this case, J (mol/cm^{2}s), is proportional to the concentration gradient (Δc) as the driving force for this process:

$$J = -D \cdot \Delta c, \tag{5}$$

where D is the diffusion coefficient in the polymer (cm^2/s), and c is the concentration of the drug within the polymer (mol/cm^3). The release rate normally depends on the time so the release kinetics is determined from:

$$\partial c/\partial t = -\Delta \cdot J = \Delta \cdot (D \cdot \Delta c) \tag{6}$$

This equation describes the transport of drug out of the hydrogel when the boundary is static (static drug delivery) [86].

The hydrogel-based dosage forms can have different designs and shapes depending on the route of drug administration (Table 4).

The topical application of hydrogels can effectively be used to deliver drugs that can help to alleviate the symptoms of many pathological conditions. For instance, Nho et al. [101] proposed a therapeutic hydrogel made of poly(vinyl alcohol) or poly(vinylpyrrolidone) for the treatment of atopic dermatitis. This product contained an extract from medicinal plants such as *Houttuynia cordata*, elm, celandine and *Canavalia gladiata*, which could be used for the treatment of dermatitis. To prepare this hydrogel poly(vinylpyrrolidone) and poly(vinyl alcohol) were dissolved in the medicinal plant extract. Then, the solution was left to set to produce a gel. It is possible to freeze/thaw the cast and introduce physical cross-links into the gel. Finally the physical gel must be treated with gamma, UV- or electron beam-radiation to initiate chemical cross-linking and to sterilize the final product. The hydrogel was supported by a hydrophilic non-woven fabric sheet and an air-permeable polyethylene film.

Furthermore, hydrogels are suitable for transdermal iontophoretic delivery of drugs, as was demonstrated in the European Patent Application EP 0 524 718 A1, where polyurethane hydrogel matrices were used as monolithic drug reservoirs. These hydrogels were synthesized from mixtures prepared by adding a prepolymer solution containing an isocyanate-capped oxyalkylene-based prepolymer in anhydrous aprotic organic solvent to water. When the organic solvent has evaporated completely, the

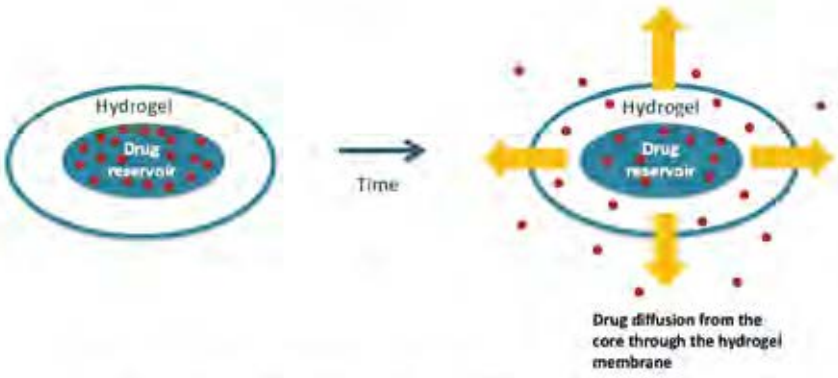


Fig. 8. Scheme of drug release through a hydrogel membrane in a reservoir system.

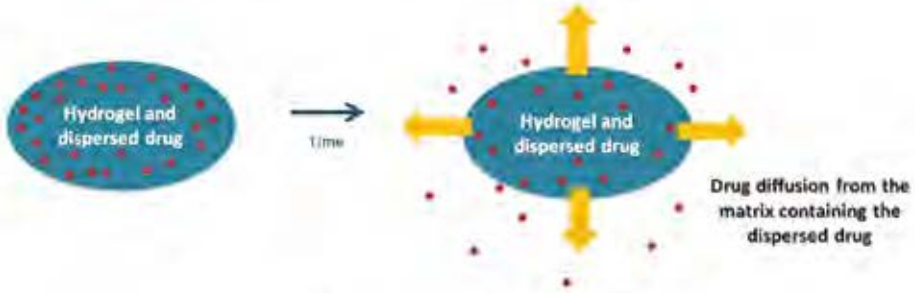


Fig. 9. Drug release from matrix systems.

Table 4
Main types of hydrogel-based products applied via different routes of drug administration.

Route of administration	Shape	Typical dimensions	References
Peroral	Spherical beads	1 μm to 1 mm	[87,88]
	Discs	Diameter of 0.8 cm and thickness of 1 mm	[89]
	Nanoparticles	10–1000 nm	[90]
Rectal	Suppositories	Conventional adult suppositories dimensions (length ≈32 mm) with a central cavity of 7 mm and wall thickness of 1.5 mm	[91]
Vaginal	Vaginal tablets	Height of 2.3 cm, width of 1.3 cm and thickness of 0.9 cm	[92]
	Torpedo-shaped pessaries	Length of 30 mm and thickness of 10 mm	[93]
Ocular	Contact lenses	Conventional dimensions (typical diameter ≈12 mm)	[95]
	Drops	Hydrogel particles present in the eye drops must be smaller than 10 μm	[96]
	Suspensions Ointments	N/A	[98]
	Circular inserts	Diameter of 2 mm and total weight of 1 mg (round shaped)	[97]
Transdermal	Dressings	Variable	[1]
Implants	Discs	Diameter of 14 mm and thickness of 0.8 mm	[98]
	Cylinders	Diameter of 3 mm and length of 3.5 cm	[99,100]

hydrogel matrix can be loaded with a drug. Transdermal iontophoresis is defined as the transport of ionic drugs through the skin, driven by a very weak electric current. The applied current helps to transfer the ionized drugs through the stratum corneum into the dermis, in which the active ingredient can diffuse into capillaries and then into the systemic circulation. Alternatively, hydrogel compositions can be employed as passive transdermal reservoirs. The hydrogels used in the aforementioned work showed a high swelling ratio, good flexibility, strength and transparency [102].

Hydrogels could be useful as ocular drug delivery carriers, not only in the form of lenses as previously discussed. The US Patent 8,409,606 B2 presented a system that provided the release of specific drugs through punctal plugs. In this work very soft biodegradable covalently cross-linked hydrogels with high-swelling capability were used, in order to be able to remain in situ (in the punctum or lacrimal canal) with greater comfort for the patient. The system could be designed to be 'temporary' or 'permanent' and the plugs could be accordingly made of collagen or silicone, respectively [103].

Ocular therapeutics™ produces ophtalmic drug delivery systems and medical devices using poly(ethylene glycol) hydrogels. For instance, dexamethasone punctum plug is designed for the controlled release of the corticosteroid in case of post-operative inflammation and pain and it has entered the Phase 3 trials. After a four-week treatment period, during which the plug releases the drug from the canaliculus to the ocular surface, it is naturally removed via the nasolacrimal system [104].

Ideally a drug delivery system should be synchronized with the physiological status of the patient and should provide drug release in response to changes in environment. Moreover, if the drug exhibits some side effects, its release when it is not required can cause additional problems. Hydrogels can show changes in their swelling behavior, structure, permeability or mechanical properties in response to various internal and external stimuli [87]. Bae et al. [105] proposed a delivery device capable of releasing a drug enclosed within a hydrogel, which deswells responding to a chemical or physical stimulation (change in temperature, pH, ionic strength or glucose concentration). It utilises either temperature- or pH- sensitive hydrogels already used in drug delivery as cross-linked homopolymers or copolymers, such as the N-isopropylacrylamide based copolymers or cross-linked weak polyelectrolytes. The system presented by Bae et al. [105] was composed of a 'sponge-like' porous gel confined in a walled structure permeable to the loaded drug. Thus, it was

possible to obtain a self-regulated drug delivery, which could be pulsatile if needed.

Biodegradable and nontoxic multi-block hydrogel copolymers have been used as drug delivery matrices and described in the US Patent 5,514,380. They were synthesized from a hydrophilic soft block and a hydrophobic, biodegradable hard block. Their degradation could be achieved with the hydrolysis of intramolecular ester and amide bond that easily occurred in the human body. Polyethyleneoxide (PEO) and/or copolymers of PEO/polypropyleneoxide (PPO) with molecular weight of 600–30,000 Da met the required qualities of the hydrophilic, non-biodegradable polymers employed in the mentioned patent. The biodegradable block could instead be represented by polylactide (PLA), polyglycolide (PGA) or a PLA/PGA copolymer [106].

US Patent 8,383,153 B2 describes a poly(amidoamine) based hydrogel for application as drug carriers. This temperature- and pH-sensitive hydrogel had a molecular structure developed to avoid the initial burst drug release and was instead capable of providing a sustained release. The material can be produced by a one-step process by coupling between secondary amine groups (—NH) of a diamine compound (such as piperazine) and vinyl groups (CH_2=CH—) of an alkylene bisacrylamide compound (e.g. N,N'-methylenebisacrylamide (MDA) or N,N'-ethylenebisacrylamide). This hydrogel can be used as a carrier for different types of physiologically active compounds, using different routes of administration [107].

A drug delivery system comprising a hydrogel and a catheter were also proposed in US Patent 7,066,904 B2. The catheter allows the incorporation and the immobilisation of a relevant amount of drug into the hydrogel, and then its release by a triggering agent or different condition in the desired location. In this case the polymers used, such as (hydroxyethyl)methacrylate-*co*-methacrylic acid, are pH-sensitive in order to produce hydrogels able to undergo a volume phase transition at a specific pH. A salt solution, such as sodium phosphate or sodium bicarbonate can be used to alter the microenvironment within the device and trigger the release of the active ingredient. In fact, the pH of this solution could be in the range of 7.5–8.4 or in the range of 6.4–7.3, and could cause alternatively swelling or contraction of the hydrogel [108].

One of the successful examples of hydrogels for drug delivery is the vaginal insert Cervidil® for cervical ripening, which has been on the market since 1995. This controlled release formulation has been used to induce or bring on labor in patients who are at or near the time of delivery. Each insert contains 10 mg of dinoprostone (prostaglandin E_2 or PGE_2) in 271 mg of cross-linked polyethylene oxide/urethane polymer and it releases the drug over a period of 12 h at approximately 0.3 mg/h. The drug release is triggered by the hydrogel swelling when placed in a moist vaginal environment [109].

Controlled Therapeutics Scotland Ltd. has developed a misoprostol vaginal insert (MVI) that uses the same delivery system as the Cervidil®, but contains misoprostol, a cytoprotective agent active on the cervix and uterus to induce labor. The same company is currently developing a modified release hydrogel buccal patch (Pilobuc™) containing pilocarpine, for the treatment of symptoms of Sjögren's syndrome, a systemic autoimmune disease in which exocrine glands that produce tears and saliva are destroyed by the immune cells [110].

A hydrogel subcutaneous insert in the form of reservoir system, called SUPPRELIN LA (Endo Pharmaceuticals Solutions Inc.), for the release of histrelin acetate is available on the market. Histrelin acetate is a gonadotropin-releasing hormone (GnRH) agonist indicated for the treatment of children with central precocious puberty (CPP). It produces a decrease in luteinizing hormone (LH) levels and sex steroids serum concentration within the first month of treatment. The implant is made of a hydrogel prepared from 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, trimethylolpropane trimethacrylate, benzoin methyl ether, Perkadox-16, Triton X-100 and contains 50 mg of histrelin, which is delivered over 12 months time (approximately 65 mcg per day). After this period the device needs to be removed as it is nonbiodegradable [111].

Park et al. [112–115] had proposed the use of superporous hydrogel compositions as gastric retentive devices for long-term oral drug delivery. These hydrogels were produced starting from (meth)acrylic acid or (meth)acrylamide, a so-called 'disintegrant', represented by a natural or synthetic cross-linked hydrophilic polymer such as cross-linked carboxymethylcellulose or poly(vinyl pyrrolidone) and a cross-linking agent such as N,N'-methylenebisacrylamide. They were synthesized using the gas blowing technique where polymerization and foaming (with sodium carbonate or bicarbonate as foaming agent) take place at the same time. More specifically, in this process the polymerization has to start only a few minutes after the beginning of foaming in order to entrap the gas bubbles in the network. The final device was able to remain in the stomach up to more than 24 h allowing the slow release of the drug loaded.

Hydrogel devices were suggested for oral delivery of different active ingredients, e.g. non-steroidal anti-inflammatory drugs (NSAIDs) [116]. They can be used to protect drugs or proteins (e.g. insulin) susceptible to the proteolytic degradation that occurs in the stomach [117,118]. In the US Patent application WO1998043615 A1 [119] a hydrogel matrix made of poly(methacrylic acid-*g*-ethylene glycol) cross-linked with tetraethylene glycol dimethacrylate is presented. This hydrogel could be loaded with insulin simply by immersing it into its solution at pH 7.4. When administered orally, insulin will be protected from the acidic environment of the stomach by the formation of inter-chain complexes within the hydrogel network. Hydrogen bonding between the carboxyl and the ether groups on the grafted chains stabilized these complexes at acidic pH. These hydrogels exhibited pH-sensitive swelling behavior: once in the upper small intestine (at higher pH), the complexes dissociate increasing the pore size and allowing the insulin to be released from the matrix. Additionally, the ability of these hydrogels to strongly adhere to the intestinal mucosa significantly improves the release and absorption of the protein [117,119].

In the future, hydrogel-based products could represent a significant proportion of drug delivery systems, to successfully administer drugs at the desired rate and site in

the body. Specific release rates and dissolution profiles could be achieved with the development of new hydrogels with different hydrophobicity/hydrophilicity and structural characteristics. These systems could improve the delivery of more sensitive molecules and be employed in the treatment of pathologic conditions such as diabetes or even cancer. Specifically, more developments are expected in the use of hydrogels for delivery of therapeutic proteins and peptides.

5. Tissue engineering

There are millions of patients suffering from the loss or failure of an organ or a tissue caused by an accident or a disease every year. Over 8 million surgeries are conducted to treat these patients in the U.S. each year, and the overall cost of these issues to the U.S. economy is estimated to be around $400 billion per year. Tissue and organ transplantations represent generally accepted therapies, but they are drammatically limited by donor shortages [120].

The term "tissue engineering" was originally defined in 1988 as the "application of the principles and methods of engineering and life sciences toward fundamental understanding of structure–function relationship in normal and pathological mammalian tissues and the development of biological substitutes for the repair or regeneration of tissue or organ function" [121]. In other words, it involves the improvement or replacement of specific tissues or organs using engineered materials and synthetic strategies.

Tissue engineering is a more recent application of hydrogels, in which they can be applied as space filling agents, as delivery vehicles for bioactive substances or as three-dimensional structures that organize cells and present stimuli to ensure the development of a required tissue (Fig. 10). Space filling agents are the most commonly used group of scaffolds and they are employed for bulking, to prevent adhesion, and as a biological 'glue'. Drugs can be delivered from hydrogel scaffolds in numerous applications including promotion of angiogenesis and encapsulation of secretory cells. Additionally, hydrogel scaffolds have also been applied to transplant cells and to engineer many tissues in the body, including cartilage, bone, and smooth muscle [122].

An indispensable property is the biocompatibility of hydrogels, which could be defined as the ability of a material to be in contact with the body organs without any damages for the surrounding tissues and without triggering any undesirable response [114]. Synthetic materials capable of forming hydrogels suitable for tissue engineering include poly(ethylene oxide), poly(vinyl alcohol), poly(acrylic acid), poly(propylene fumarate-co-ethylene glycol), and polypeptides. Agarose, alginate, chitosan, collagen, fibrin, gelatin, and hyaluronic acid are naturally derived polymers that could also be used for this purpose [124,125].

In European patent EP 1 664 168 B1, an interesting hydrogel-based composition for manufacturing porous scaffolds has been presented. It was composed of a biodegradable unsatured self-cross-linkable polymer such as poly(propylene fumarate), biodegradable hydrogel microparticles (diameters of 1–1000 μm) entrapping water and a free-radical initiator promoting the cross-linking process. The microparticles were made of cross-linked collagen or gelatin and can contain a biologically active substance. The method disclosed 'super-absorbent semi-solid' hydrogel microparticles, able to swell in water but not to flow as a liquid, with a defined shape due to the cross-linking. After the polymerization process, the scaffold formed with the mixture could be used directly for the treatment of skeletal defects without leaching out the hydrogel porogen [125].

Harris et al. [126] described a tissue engineering scaffold with the benefits of microporous and nanoporous scaffolds, comprising a nanofibrous and nanoporous hydrogel formed from self-assembling peptides, which are

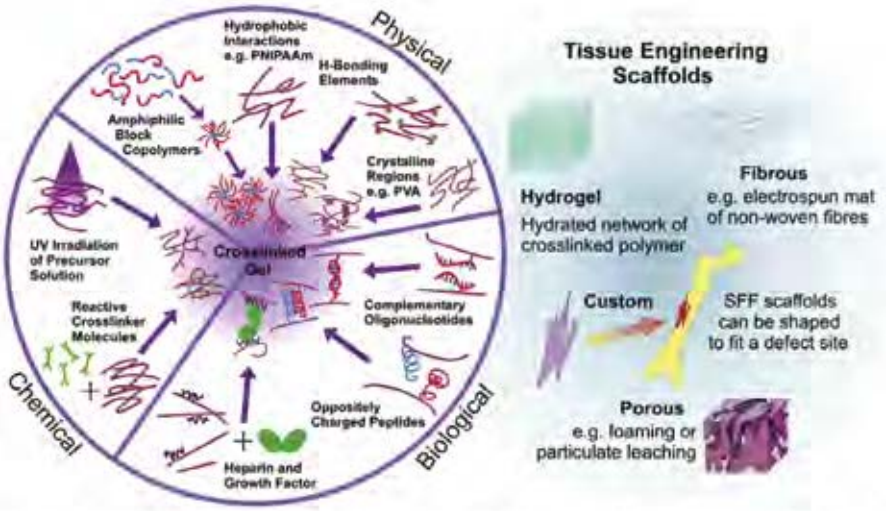


Fig. 10. Hydrogels in tissue engineering (reprinted from E.S. Place, J.H. George, C.K. Williams, M.M. Stevens, Chem. Soc. Rev. 2009, 38, 1139–1151 [123] with permission from the Royal Society of Chemistry).

non-immunogenic, biodegradable, and capable to interact with cells. They were able to stimulate tissue ingrowth and vascularization, and furthermore, this hydrogel could be used for slow-diffusion drug delivery. The self-assembling peptides used to form hydrogels should have alternating hydrophobic and hydrophilic amino acids (more than 8). For instance, one of them had the following amino acid sequence: Arg–Ala–Asp–Ala–Arg–Ala–Asp–Ala–Arg–Ala–Asp–Ala–Arg–Ala–Asp–Ala. This peptide is commercialized as 'PURAMATRIX' (3-D Matrix, Inc., Cambridge, Mass.) Its self-assembly could easily take place in tissue culture medium (Dulbecco Modified Eagle's Medium, Gibco BRL, Gaithersburg, Md) containing calf serum. The scaffolds presented might also be applied to open wounds or be surgically implanted. It was established that scaffolds made of only one component or phase may not produce the ideal environment for supporting tissue regeneration. Conversely, the so-called 'hybrid' materials were found to give better results, in terms of cell proliferation, differentiation and migration.

Hydrogels scaffolds are used for cell-sheet and tissue production. Kumar [127] has recently disclosed a method to produce biodegradable poly(vinyl alcohol) hydrogels complexed with phenylboronate-containing polymers able to encourage cell and tissue growth. PCCs include a phenylboronate ligand (such as 4-vinylphenylboronic acid), an acrylic monomer (such as N-isopropylacrylamide or acrylic acid) and an alkaline tertiary amine (such as N,N-dimethylaminoethylmethacrylate). The cells which could be represented for example by keratinocytes or fibroblasts, are cultured for 5–20 days on the hydrogel scaffolds. It is then possible to collect the cell layers formed by simply dissolving the hydrogel scaffolds using a saccharide solution (such as fructose or mannitol solution). This saccharide biodegradation is possible due to the presence of phenylboronate ligands that are derivatized forms of phenylboronic acid, which can establish reversible covalent interactions with 1,2 or 1,3-cis-diol-containing compounds such as carbohydrates.

Blanchard et al. [128] has reported the use of pure cross-linked keratin-based hydrogels for tissue engineering cell scaffolds. Keratin is biocompatible, and non-immunogenic biopolymer that promotes epithelialization process and can be extracted from patient hair or nails. After purification and partial oxidization of the keratin, the sulfonic acid residues of the protein, which are hydrophilic, form disulfide cross-links between backbones and bind water. Additional hydrogen bonds are then formed in this hydrogel. The material was shown to be suitable as nutrient support and scaffold for cell growth.

Song et al. [129] has proposed beta-glucan-based hydrogel scaffolds for tissue engineering produced by radiation fusion technology. Beta-glucan (beta-1,6-branched-beta-1,3-glucan) can promote cell regeneration and collagen biosynthesis, and it is recognized to be safe and biocompatible. It could be extracted from different fungi such as *Schizophyllum commune* or *Ganoderma lucidum* and dissolved in distilled water. This aqueous solution was then cast in petri dishes and irradiated for the cross-linking step using electron, gamma or UV beam at a dose of 5–50 kGy to form a gel. Stem cells could rapidly adhere, grow and differentiate on the scaffold formed.

One of the most important future challenges in tissue engineering is how polymers could be used to stimulate the blood vessel network formation in the desired tissue, essential to supply its needs. Hydrogels could represent a valid option to effectively control the vascularization process, by local delivery of both angiogenic factor and endothelial cells to the intended area [120]. Additionally, many types of tissue such as bone, muscle or blood vessels are located in areas requiring excellent mechanical properties that the majority of the currently available hydrogels do not show, so new approaches should be investigated in the future to achieve better results.

6. Hygiene products

Superabsorbent polymers (SAPs) have been introduced into the agriculture and diaper industry about thirty years ago, and since then their uses have been extended to several other applications due to their excellent water retention [130]. SAPs have been firstly commercially produced in Japan in 1978 for use in feminine napkins, and this early material was represented by a cross-linked starch-g-polyacrylate [131].

At the end of the 90s, 'superporous hydrogels' (SPHs) were introduced and presented as a different type of water-absorbent polymer system. As SAPs, SPHs are formed by covalently cross-linked hydrophilic polymers, but unlike SAPs, they show an exceptional size-independent fast swelling kinetics. The first generation of SPHs was generally made from highly hydrophilic acrylamide, salts of acrylic acid and sulfopropyl acrylate. Later generation of SPHs are represented by 'hydrid SPHs' produced by adding a so-called 'hybrid agent' (natural or synthetic water-soluble or dispersible polymer capable of chemical or physical cross-linking) to the SPH previously made. With this method it is possible to generate an interpenetrating polymeric network. For example, acrylamide-based SPH is synthesized in the presence of sodium alginate and after that, a cross-linking occurs between alginate chains and calcium ions forming a 'hybrid SPH'. These more recent SPHs have shown better and more useful qualities, such as high mechanical strength and elasticity even in swollen state [130]. Superabsorbent hydrogels, in particular the acrylate-based materials, are extensively used in hygiene products to absorb fluids. In fact they are able to hold moisture away from the skin, promoting skin health, preventing diaper rash and providing a comfortable use. Parents in all the industrialized countries as well as hospitals around the world employ disposable diapers containing SAPs [132].

A further increase in the use of these materials is observed in training pants and adult incontinence product markets. SAPs can also prevent the colonization of germs, reducing the risk of fecal contaminations and potential spread of gastrointestinal infections. The first use of SAPs in the diaper industry was proposed in 1982 by Unicharm in Japan, with its subsequent use in sanitary napkins. After that, diapers became thinner and also had improved water

retention performance. It was possible to develop diapers with leakage values below 2% and the standard weight of a medium size diaper could be reduced by about 50%, with some obvious advantages in terms of environmental issues and reduced manufacturing costs [132].

Regarding the ecological impact of disposable diapers and similar products, it is relevant to consider current diaper consumption. For instance, a child within the 30th month uses approximately six diapers a day and each of them has a volume of 500 cm^3, so only one child produces on average 3000 cm^3 of litter a day, i.e. 1092 cubic meters every year [132]. Making recyclable disposable diapers, napkins, hospital bed sheets, sanitary towels and other similar products is therefore one of the vital targets for the modern industry. An innovative solution to this problem has recently been proposed, which involves the use of cellulose-based hydrogels, which are totally biodegradable. Novel types of hydrogels, containing sodium carboxymethylcellulose (NaCMC) and hydroxyethyl cellulose (HEC) cross-linked with divinyl sulfone (DVS), can swell like SAPs, and exhibit high water retention under centrifugal loads. These improvements were achieved by introducing microporous structures into the hydrogel, which increases water retention and swelling kinetics due to capillarity effects [132].

US Patent 32,649 describes one of the first hydrogel-forming polymer compositions suitable for hygiene products manufacturing. It consisted of a water-insoluble, slightly cross-linked polymeric material, which could be prepared from carboxylic acids and acid anhydrides, or olefinically unsatured sulfonic acids, using a free-radical polymerization in the presence of a cross-linking agent in an aqueous solution. This material could be dried to result in polymer compositions capable to form hydrogels upon contact with water or bodily fluids [133]. Only a few years later, in US Patent 5,009,653, Osborn proposed a product consisting of a thin and flexible sanitary feminine napkin with an absorbent core placed between two air-laid tissue sheets. The core was composed of a hydrogel-forming material, prepared from acidic monomers such as acrylic acid, methacrylic acid or 2-acrylamido-2-methyl propane sulfonic acid. This material was highly absorbent, could withstand medium to high menstrual flows and was very conformable to the body of a user, preventing the risk of leakage and staining [134].

Many attempts have been made to develop new products, which could not only swell, but also retain the fluids absorbed under external pressure or against an applied restraining force. An absorbent material composed of a porous matrix of fibers and superabsorbent hydrogel is described in the US Patent 5,147,343, which has the capability to initially imbibe fluids and swell, while being exposed to a load. The matrix can be formed from wood pulp or cotton linters as well as synthetic fibers (polyethylene, polypropylene polyesters etc.) and the hydrogel could be produced from polyacrylamides, polyvinyl alcohol, ethylene-maleic anhydride copolymers or polyvinyl ethers. The 'Absorbency Under Load' (AUL) is defined as the volume of 0.9 wt% NaCl solution which the superabsorbent composition could absorb per 1 g in one hour, being subjected to a load of 21,000 dynes/cm^2. Hence, the work (W) performed by the material could be calculated using the following formula [135]:

$$W = (\text{AUL}) \times (\text{Restraining force}) \quad (7)$$

Pampers (owned by Procter & Gamble) and Huggies (from Kimberly–Clark) are the two most widely used disposable diaper brands, with about 35% and 22% global market share, respectively. Both are sold in over 50 countries and they have wide range of products. Manufacturers have been focusing their efforts on enhancing the production and engineering of SAPs with better properties, i.e. higher AUL, lower levels of residual monomers (RM) and soluble fractions [136]. Further developments in this area are expected with the formulation of the materials containing enzymes and other additives to prevent infections and unpleasant smells. Additionally, taking the scale of production of these materials into consideration, there is a clear need in environmentally friendly hygiene products that undergo biodegradation.

7. Conclusions

Hydrogels are widely present in everyday products though their potential has not been fully explored yet. These materials already have a well-established role in contact lenses, hygiene products and wound dressing markets but commercial hydrogel products in tissue engineering and drug delivery are still limited. Many hydrogel-based drug delivery devices and scaffolds have been designed, studied and in some cases even patented, however not many have reached the market. More progress is expected in these two areas. Limited commercial products with hydrogels in drug delivery and tissue engineering are related to some extent to their high production costs.

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Voice outcomes of polyacrylamide hydrogel injection laryngoplasty.

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The Laryngoscope
Lippincott Williams & Wilkins

Voice Outcomes of Polyacrylamide Hydrogel Injection Laryngoplasty

Seung Won Lee, MD; Young-Ik Son, MD; Chan Hee Kim, MD; Jae Yong Lee, MD; Shi Chan Kim, MD; Yoon Woo Koh, MD

Objectives: Polyacrylamide hydrogel (PAAG, Aquamid) is widely used as permanent facial tissue filler during facial plastic surgery. In this study, we examined the long-term effects and safety aspects of PAAG as a vocal fold augmentation material for patients with permanent unilateral vocal cord paralysis.

Study Design: Prospective clinical trials.

Methods: PAAG injection laryngoplasty was performed in 34 consecutive patients with permanent unilateral vocal cord paralysis. Percutaneous injection was performed under local anesthesia into the vocalis muscle using disposable 25 gauge long needles. Of the 34 patients, 16 completed acoustic, perceptual, stroboscopic, and subjective evaluations prior to the injection and at 6 and 12 months after the injection.

Results: Acoustic and perceptual parameters (GRBAS [Overall grade of dysphonia, Roughness, Breathiness, Aesthenia, Strain], Maximal phonation time [MPT], jitter, and shimmer) were significantly improved ($P < .05$) after injection and remained stable over 12 months. The grades of mucosal waves and glottic closure were also significantly improved ($P < .01$). The voice handicap index (VHI), as well as the visual analogue scale (VAS) of hoarseness and aspiration significantly improved over 12 months. No adverse effects were observed except for a decrease in the mucosal wave of one patient, after injection into a superficial area of the vocal fold.

Conclusion: Based on the preliminary results of this trial, PAAG appears to be a long-lasting and safe injection material that is suitable for the treatment of glottal insufficiency caused by permanent unilateral vocal cord paralysis.

Key Words: Injection laryngoplasty, polyacrylamide hydrogel, vocal cord paralysis.

***Laryngoscope*, 117:1871–1875, 2007**

INTRODUCTION

In recent years, injection laryngoplasty techniques have regained popularity as means to manage unilateral vocal cord paralysis.[1] Injection laryngoplasty is easier and less invasive than medialization thyroplasty and may provide equally durable and effective results.[2] In Korea, hyaluronic acid (Rofilan, Rofil Medical International, Breda, The Netherlands) and collagen–polymethylmethacrylate suspension (Artecoll, Rofil Medical International, Breda, The Netherlands) are the most commonly used injection materials. These materials are highly biocompatible and biologically stable, and can yield good postoperative voice function. However, the resorption of injected materials over varying periods of time is problematic, especially for patients with permanent unilateral vocal cord paralysis.

Polyacrylamide hydrogel (Aquamid, Ferrosan AS, Soeborg, Denmark) is a new space filler for esthetic facial correction that lasts longer than other space fillers. It is homogenous, stable, non-biodegradable, and has optimal viscosity and elasticity in human facial tissue. PAAG represents a good alternative material for injection laryngoplasty for the treatment of permanent vocal cord paralysis. However, vocal-fold injection is still considered an off-label application due to the lack of controlled clinical trials.

We present the results of the first trials of a prospective-type pilot study to determine the safety and usefulness of PAAG injection laryngoplasty in the treatment of patients with permanent unilateral vocal cord paralysis.

MATERIALS AND METHODS

Patients

Among 34 consecutive patients with permanent unilateral vocal cord paralysis, 16 were enrolled for this study. They received PAAG injection laryngoplasty between January 2005 and December 2006 at the Department of Otolaryngology–Head and Neck Surgery, Soonchunhyang University Bucheon Hospital. Exclusion criteria were as follows: five patients had incomplete follow-up data, seven patients had an insufficient observation period, and six patients died from their cancer before all the data could be collected. Postoperative follow-up periods ranged from 12 to 22 months (mean 15.6 ± 3.2 months). The final sample group included 12 men and 4 women, with a mean age of 64.6 years (range 33–78 years).

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TABLE I.
Summary of Patients' Primary Cause of Unilateral Vocal Cord Paralysis (n = 16).

Cause	Number of Patients Affected, n (%)
Lung cancer	5 (31.3)
Esophageal cancer	4 (25.0)
RLN section during thyroidectomy	3 (18.8)
Advanced thyroid cancer	2 (12.5)
Pneumonectomy	1 (6.3)
Pulmonary tuberculosis	1 (6.3)
Miscellaneous	2 (12.5)

RLN = recurrent laryngeal nerve.

The inclusion criteria were as follows:

1. Patients with terminal cancer that was the cause of their vocal cord paralysis from direct tracheoesophageal groove invasion, and metastatic lymph node involvement for recurrent laryngeal nerve course—advanced lung cancer (five cases), esophageal cancer,[4] thyroid cancer;[2]
2. Patients in whom the recurrent laryngeal nerves had been sacrificed during thyroidectomy because of evident thyroid cancer invasion;[3]
3. Patients who had been diagnosed with permanent vocal cord paralysis that was confirmed by laryngeal electromyography (EMG)–pneumonectomy,[1] pulmonary tuberculosis.[1]

Table I summarizes the patients' characteristics.

Objective Voice Evaluation

Acoustic and aerodynamic analyses were followed up prospectively before injection and at 6 and 12 months after injection. For the acoustic analyses, each patient sat in a quiet room wearing a microphone (AKG, C410, Vienna, Austria) at a constant 3-cm distance from the mouth. Sustained 3-second /Ah/-like phonations were recorded, and jitter and shimmer were analyzed using Dr. Speech 4.0 software (Tiger DRS Inc., Seattle, WA).

For aerodynamic analyses, the maximal phonation time was calculated. Each patient sustained /a/ vowel phonations for as long as possible over three trials.

Subjective Voice Evaluation

Perceptual voice evaluations were conducted using the GRBAS (Grade, Roughness, Breathiness, Asthenia, Strain) rating scale. The five parameters evaluated were overall grade of dysphonia, roughness, breathiness, asthenia, and strain. Professional speech language pathologists evaluated voice quality using the GRBAS scale, especially for overall grade and breathiness (0 = normal; 1 = slight disturbance; 2 = moderate disturbance, and 3 = severe disturbance). A 10-point visual analogue scale (VAS) was applied to the patients who self-rated their voice function and the degree of aspiration independently (0 = normal voice/no aspiration problems; 10 = worst voice/maximal aspiration problems) to evaluate subjective voice improvement and aspiration status. None of the patients received pre- or postinjection voice therapy or antibiotics.

For videostrobolaryngoscopic evaluation, each patient sustained /i/ like phonations at his or her habitual pitch, which was recorded using a rigid endoscope (Rhinolaryngeal stroboscope 9100, Kay Elemetrics, Lincoln Park, NJ). Mucosal wave and glottic closure were also analyzed during the stroboscopic evaluation.

For practical purposes, we rated the mucosal wave of vocal-fold vibration and the glottic closure independently using a four-point grading scale (0 = no wave, wide open; 1 = marked decreased mucosal wave, large glottic gap; 2 = slightly decreased mucosal wave, small glottic gap; and 3 = full wave, complete closure).

At each follow-up visit, the patients also completed the Voice Handicap Index (VHI), which is a patient-based survey divided into three subscales that measure the functional, physical, and emotional aspects of the handicap caused by voice impairment. Subscale scores can range from 0 to 40, and total scores can range from 0 to 120; a higher score indicates a greater degree of handicap. None of the patients received voice therapy or antibiotics after injection.

Injection Procedures

All PAAG injections were performed percutaneously under local anesthesia by a junior surgeon. Percutaneous vocal fold injections were administered through the cricothyroid membrane directly into the vocalis muscle using a disposable 25 gauge (G) long needle under transnasal flexible fiberscopic (Olympus laryngobronchoscope type T3, Tokyo, Japan) monitoring. Before entering the operating room, each patient received a small amount of intravenous sedative and inhaled 4% lidocaine nebulizer for 10 minutes. An extra nozzle was used to spray additional 4% lidocaine onto the pharynx, larynx, and nose. When the needle reached the vocal fold, the needle was positioned correctly by moving its tip up and down. Injections were usually administered

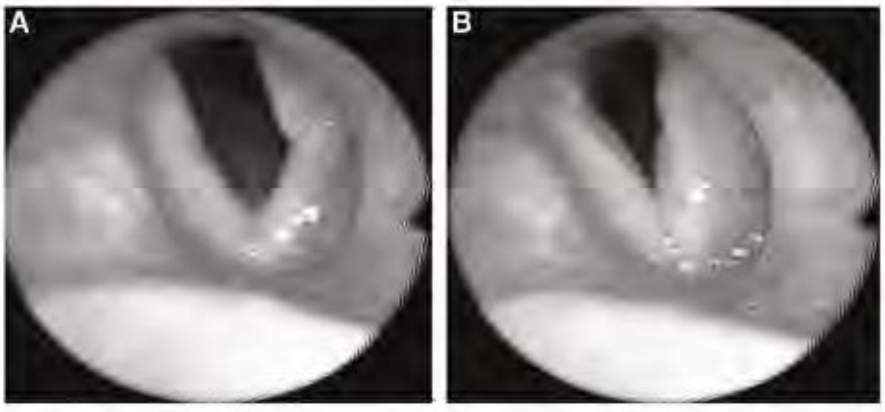

Fig. 1. Intraoperative fiberscopic views of Polyacrylamide hydrogel (PAAG) injection laryngoplasty. (A) Preoperative view. The left immobile vocal fold shows paramedian position prior to injection. (B) The left vocal fold shows some overcorrection after injection.

TABLE II.
Demographic Data and Results of Voice Analyses Performed Preoperatively and at 6 and 12 Months Postoperative.

					Preoperative				6 Months Postoperative				12 Months Postoperative				
No.	Sex	Age	Site	Cause	MPT	VHI	Jitter	Shim	MPT	VHI	Jitter	Shim	MPT	VHI	Jitter	Shim	Comment
1	M	71	R	TC	2.40	58	2.23	9.75	4.28	25	2.47	5.57	3.47	5	1.41	10.33	
2	M	78	L	EC	7.58	44	1.94	7.97	14.5	16	0.75	6.51	14.71	6	1.33	4.38	
3	M	72	R	EC	3.79	45	3.21	8.93	5.23	21	1.94	3.05	5.39	11	1.94	3.05	
4	M	51	L	LC	3.72	65	2.81	14.59	8.99	42	0.25	2.63	10.21	12	1.58	3.86	
5	F	77	L	LC	2.88	76	3.20	5.20	6.37	22	0.23	1.33	8.90	20	0.21	1.11	
6	M	63	L	TS	7.27	46	1.32	9.40	9.03	23	1.44	9.19	8.52	22	7.52	12.57	
7	M	75	L	LC	3.98	61	2.31	7.97	5.52	29	1.81	5.98	3.35	29	0.96	4.24	
8	F	73	R	TC	2.95	104	2.85	8.80	5.54	35	1.95	5.80	5.45	32	1.30	4.18	Booster
9	M	41	L	EC	6.87	54	2.35	11.06	8.69	45	1.35	1.56	5.92	34	0.32	1.41	
10	M	75	R	THY	4.45	98	0.44	4.13	5.32	90	0.76	2.40	6.21	35	1.78	3.25	
11	M	59	L	THY	2.74	53	3.41	9.74	4.84	39	0.80	5.60	5.50	39	0.71	4.44	
12	M	61	L	TBC	4.17	51	0.40	1.42	8.90	41	0.10	0.40	8.72	50	0.19	0.86	
13	F	33	R	THY	3.23	81	0.89	3.24	5.90	52	0.24	1.61	7.84	52	0.53	2.47	
14	M	73	L	LC	4.09	85	4.35	6.82	6.64	52	2.64	4.74	5.89	68	2.10	5.89	
15	M	69	R	EC	5.66	56	0.22	2.30	6.72	21	0.78	3.99	5.74	74	0.78	3.99	Dec mw
16	F	63	L	LC	6.73	97	0.32	2.16	8.66	82	0.30	1.98	8.42	92	0.51	2.92	
Average		64.6			4.53	67.3	2.08	7.09	7.10	39.3	1.11	3.77	7.13	36.4	1.44	4.31	

TC = thyroid cancer; THY = recurrent laryngeal nerve sacrificed during thyroidectomy; EC = esophageal cancer; LC = lung cancer; TS = thoracic surgery; TBC = pulmonary tuberculosis; MPT = maximal phonation time (sec); VHI = Voice Handicap Index; Jitter = percentage of jitter; Shim = percentage of shimmer; Booster = booster injection; Dec mw = decrease glottic mucosal wave.

to the vocalis muscle in front of the vocal process and continued until a slight overcorrection was achieved (Fig. 1).

We overcorrected the paralyzed vocal fold based on estimates of postoperative carrier resorption and consequent infiltration into the surrounding tissue (15% to 20%). The mean volume of PAAG injected into each patient was 0.85 mL (range 0.4–1.2 mL).

Statistical Analysis

Statistical analyses (SPSS 10.0 for Windows, Chicago, IL) were performed using the Wilcoxon signed-rank test; P values less than 0.05 were considered statistically significant.

RESULTS

Objective Parameters

Table II presents the demographics and voice analysis data. Among the 34 patients, 16 fully completed the acoustic and aerodynamic analyses. Statistical increases were observed in maximal phonation time, and statistical decreases were observed in jitter and shimmer during the postoperative 12-month follow-up. The average maximal phonation time increased significantly from 4.53 ± 1.73 seconds to 7.10 ± 2.60 seconds at 6 months and to 7.13 ± 2.81 seconds at 12 months ($P < .01$). The average percentage of jitter decreased from 2.06% ± 1.27% to 1.11% ± 0.84% at 6 months and to 1.44% ± 1.73% at 12 months ($P < .05$). The average percentage of shimmer decreased from 7.09% ± 3.70% to 3.77% ± 2.32% at 6 months and to 4.31% ± 3.11% at 12 months ($P < .01$) (Fig. 2).

Subjective Parameters

The patients' overall ratings of their symptomatic voice function and aspiration status using VAS revealed

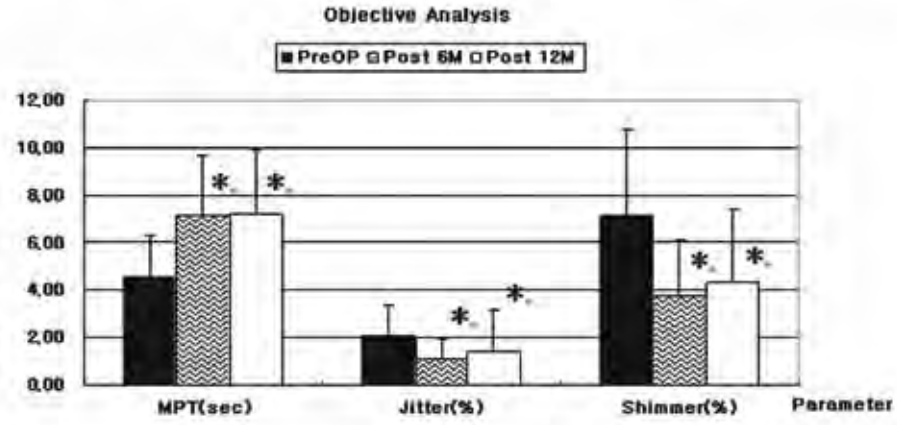

Fig. 2. Preoperative maximal phonation time, shimmer, and jitter are significantly improved at postoperative months 6 and 12. *Significant improvement compared to prior injection.

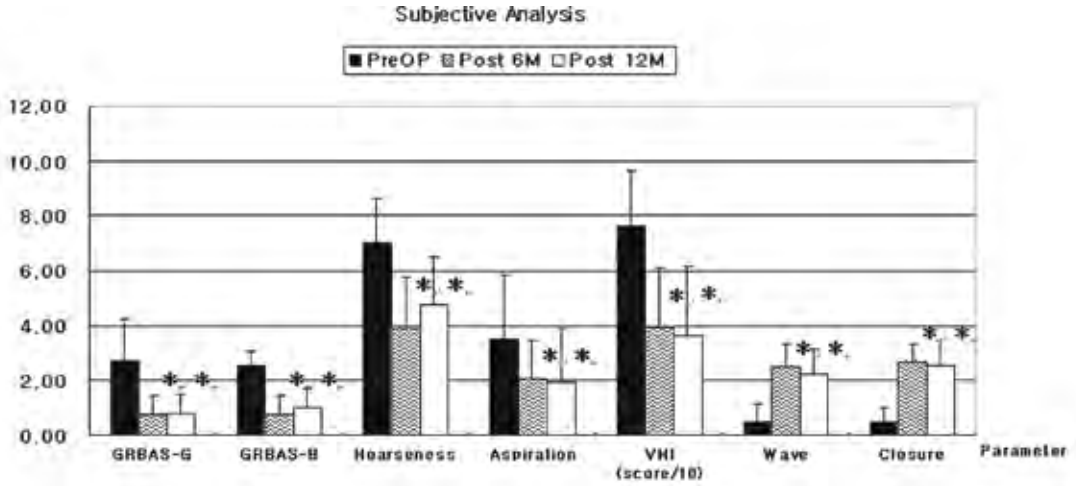

Fig. 3. Preoperative GRBAS scale, visual analogue scale (VAS) of voice function (hoarseness) and the degree of aspiration, Voice Handicap Index (VHI), and grades of mucosal wave of vocal fold vibration and glottal closure show significantly improvements at postoperative months 6 and 12. *Significant improvement compared to prior injection.

significant improvements over their baseline evaluations. The average voice VAS scale improved from 7.00 to 3.88 at 6 months and to 4.75 at 12 months ($P < .01$). The average aspiration VAS scale improved from 3.50 to 2.06 at 6 months and to 1.94 at 12 months ($P < .05$).

The patients' VHI values improved significantly from 67.25 ± 20.18 to 39.31 ± 21.88 at 6 months and to 36.44 ± 25.21 at 12 months ($P < .01$).

The GRBAS scale also revealed significant improvement of overall grades of dysphonia (G) and breathiness (B) during the 12-month follow-up ($P < .01$).

Stroboscopic analyses revealed that the average grades of mucosal wave and glottal closure significantly improved and remained stable over the 12-month period.

The average grade of mucosal wave improved significantly from 0.50 to 2.50 at 6 months and to 2.25 at 12 months ($P < .01$). The grade of glottal closure pattern improved significantly from 0.50 to 2.69 at 6 months and to 2.56 at 12 months ($P < .01$) (Fig. 3).

Postoperative Complications

No serious adverse effects, such as airway obstruction, material migration, or granuloma formation, were observed in any of the patients during the follow-up period.

One patient demonstrated minimal mucosal erythema and edema at the site of injection that may have resulted from a traumatic needle-tip injury to the vocal fold during injection. However, this resolved spontaneously without any problems.

Another complication was caused in one patient by failure to augment the paralyzed vocal fold, since PAAG leakage occurred after puncturing the vocal fold mucosa during the injection. We treated this patient using booster injection laryngoplasty at postoperative 1 month.

The most serious complication observed during the study was a decrease in the mucosal wave of one patient after injection into a superficial area of the vocal fold. Although this patient was satisfied with his voice quality, his mucosal wave was diminished as compared to the other injected patients. However, his vocal fold vibration gradually improved over 12 months.

DISCUSSION

Unilateral vocal cord paralysis usually causes breathiness during phonation, together with considerable aspiration problems. The primary purpose of the phonosurgical procedure is to enhance quality of life by creating a more effective and understandable voice.[2]

Injection laryngoplasty is the least invasive way of increasing vocal fold volume to obtain glottic closure in cases of glottic incompetence.[3] This technique can be accomplished transorally or percutaneously, usually under local anesthesia or under suspension microscopy.[3] However, patients who are candidates for injection laryngoplasty tend to have many comorbid conditions that increase their risk for complications during surgery while under general anesthesia. In addition, if vocal cord paralysis occurs during surgery, any additional general anesthesia can be very stressful for patients for psychological, physical, and financial reasons. Despite the technical difficulties, we performed injection laryngoplasty under local anesthesia via the cricothyroid membrane.

Because of technological advances, many new materials have become available for injection laryngoplasty.[4] Various highly biocompatible materials, such as bovine collagen, micronized human dermis, and hyaluronic acid derivatives, are now widely applied.[4–6] In Korea, hyaluronic acid (Rofilan, and Restylene, Q-med, Upsala, Sweden) and collagen–polymethylmethacrylate suspension (Artecoll) are commonly used for injection laryngoplasty. These materials are highly biocompatible, biologically stable, and reportedly yield good postoperative voice function.[7,8,11]

The resorption of injected materials over varying periods of time can be problematic, often necessitating repeated injections, especially in patients with permanent unilateral vocal cord paralysis. Among the available space

fillers, hyaluronic acid is classified as a resorbable material, PMMA as a semipermanent material, and PAAG as a permanent material.[14]

PAAG is one of a new generation of long-lasting injectable space fillers, and it lasts longer than other available space fillers. PAAG consists of approximately 2.5% cross-linked polyacrylamide polymer and 97.5% nonpyogenic water.[15] It is known to be homogenous, stable, and non-biodegradable and to exhibit optimal viscosity and elasticity in human facial tissue.[12]

For more than 10 years, PAAG has been used extensively throughout Eastern Europe under various trade names to augment soft tissue defects; it was authorized for sale in Europe in March, 2001 (Conformity of European Mark No. 0543). Because of its larger molecular size, it does not migrate through human tissues and can be maintained over several years.[13]

Another benefit of PAAG is that it is readily available without any preparation. Its low viscosity allows it to be injected through a simple syringe and needle (27G or larger) without any special equipment such as a Breuning injector. The improved ease of injection has allowed the introduction of percutaneous administration in an office setting, thereby avoiding general anesthesia and hospital admission.

PAAG appears to be a viable alternative material for injection laryngoplasty, especially in the treatment of permanent vocal cord paralysis. Since it does not cause a foreign body reaction in human facial tissues such as lip, forehead, nasolabial cleft, or nasal area,[12,13] PAAG retains its effects longer than other materials. This characteristic makes it unnecessary to administer repetitive vocal fold injections for the treatment of permanent vocal cord paralysis. However, because of the lack of controlled clinical trials, vocal fold injection of PAAG is still considered an off-label application. The present study represents the first prospective pilot study to determine the safety and usefulness of PAAG injection laryngoplasty for patients with permanent unilateral vocal cord paralysis. Given the study characteristics, we included only patients with vocal cord paralysis caused by terminal cancer, i.e., esophageal cancer or lung cancer, and those patients who had direct recurrent laryngeal nerve invasion by thyroid cancer.

Most of the study participants were subjected to chemotherapy for their cancer. It proved difficult for the patients to follow a regular diet because of oral mucositis caused by the chemotherapeutic agent, aspiration from incomplete glottic closure, and voice impairment. In this situation, injection laryngoplasty using permanent material under local anesthesia in the office setting can improve the quality of life of patients during cancer treatment.

Our study reveals that PAAG injection laryngoplasty confers statistically significant improvements in voice function and aspiration, and that therapeutic efficacy continues for at least 12 months. These results are supported by the aerodynamic, perceptual, stroboscopic, VHI, and VAS results. The vibratory activity of the vocal fold and its closure pattern also improved in all but one of the patients, and these results were confirmed in the stroboscopic follow-up. The effectiveness of PAAG may persist for several years or more, although long-term follow up will be required.

Our results also confirm that PAAG injection has low morbidity. In the injected patients, only two minor complications were noted: mild erythema of the injected vocal fold, and PAAG leakage because of vocal fold mucosa perforation. However, superficial injection of PAAG could cause serious complications as it is regarded as a long-lasting material. In this situation, PAAG may be removed under general anesthesia. Thus, superficial injection of PAAG should be avoided for appropriated vocal fold vibration.

More clinical studies and long-term follow-up periods are required to investigate this problem. We plan to establish an animal model to study the long-term effects of PAAG injection on vocal folds.

CONCLUSIONS

Based on the preliminary results of this trial, PAAG injection laryngoplasty is able to improve the voices and voice-specific quality of life of patients with permanent unilateral vocal cord paralysis. Vocal fold injection with PAAG is a convenient, safe, and useful method for treating glottal insufficiency caused by permanent unilateral vocal cord paralysis.

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PART II:

OTHER RECENT INVESTIGATIONS CONCERNING OA CLINICAL TRIALS & PAAG

CHAPTER 1

LASTING EFFECT

TNIBAR et al.

An international multi-centre study on the efficacy of an intraarticular polyacrylamide hydrogel in horses with osteoarthritis: a 24 months follow–up.

Acta Veterinaria Scandinavica (2015) 5:20

TNIBAR et al.

An international multi-centre one year prospective study on the efficacy of an intraarticular polyacrylamide hydrogel in horses with osteoarthritis.

Proceedings icrs (2012, Turkey)

TNIBAR et al.

Efficacy of a polyacrylamide hydrogel in horses with symptomatic osteoarthritis: an international multi-centre prospective study.

Equine Veterinary Journal - EVJ 44 suppl. 39 (2012) 2-18

LOWE et al.

Intra-articular 2.5% polyacrylamide hydrogel (PAAG): A prospective study on 54 thoroughbred racehorses.

39th Bain fallon memorial lectures conference, Annual equine veterinary congress, Posters (2016, Australia)

TNIBAR et al.

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Acta Veterinaria Scandinavica (2015) 5:20

Tnibar et al. Acta Veterinaria Scandinavica (2015) 57:20
DOI 10.1186/s13028-015-0110-6

AVS ACTA VETERINARIA SCANDINAVICA

RESEARCH Open Access

An international multi-centre prospective study on the efficacy of an intraarticular polyacrylamide hydrogel in horses with osteoarthritis: a 24 months follow-up

Aziz Tnibar[1*], Hans Schougaard[2], Linus Camitz[3], Jonas Rasmussen[4], Marc Koene[5], Werner Jahn[6] and Bo Markussen[7]

Abstract

Background: Polyacrylamide hydrogel (PAAG) was evaluated recently to treat osteoarthritis (OA) in horses with highly encouraging results; however no long term field-study was done to explore its clinical efficacy and lasting effect. The objective of this study was to evaluate the efficacy of PAAG in improving clinical signs of OA in horses. We hypothesized that lameness grade would significantly improve and the effect would last at least 2 years in osteoarthritic joints treated with PAAG. Forty three horses older than 2 years with OA in only one joint based on clinical evaluation, intra-articular anaesthesia and imaging (radiography) were included in this study. Horses were injected with 2 ml of PAAG into the affected joint and were followed up at 1, 3, 6, 12 and 24 months. Efficacy of PAAG was evaluated by blinded clinical assessment of lameness. Adverse reactions to joint injection were assessed. Data relating to case details, type of activity, joint and limb involved, lameness duration, lameness grading, previous joint treatment, joint effusion grading, radiographic grading, and owner assessment were recorded. Factors associated with the outcome measure "lameness grading" were analyzed using generalized linear mixed model for logistic regression.

Results: At 1, 3, 6, 12 and 24 months follow-up, 59%, 69%, 79%, 81/% and 82.5% of horses were non-lame respectively. Reduction of joint effusion was observed over time. No side effect was observed in the treated joints. There was a significant decrease in lameness grade from baseline to 1, 3, 6, 12 and 24 months ($P < 0.0001$) and a significant positive association with joint effusion ($P < 0.0001$). Estimates for odds ratio (OR) showed that the effect of treatment increased over time (OR for lower lameness from month 1 to month 24 relative to baseline increased from 20 to 58).

Conclusions: PAAG significantly alleviated lameness and joint effusion in osteoarthritic joints. PAAG is a safe and lasting (at least 24 months) OA treatment in horses. PAAG is a promising new treatment for OA in horses.

Keywords: Osteoarthritis, Horse, Treatment, Polyacrylamide hydrogel, 2 years follow up

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Background

Osteoarthritis (OA) is a common clinical problem in horses [1,2] and is the most common joint disease and one of the most frequent causes of physical impairment in humans [3]. Surveys estimate that up to 60% of lameness problems in horses are related to OA [1], which can occur early in the equine athlete's career or later in older horses [4]. The fetlock (metacarpo(tarso)phalangeal) joint is a common joint for spontaneous OA in horses [5].

As part of the OA-complex, elastoviscosity of the synovial fluid is abnormally low [6], and thus the use of visco-supplementation, for example intra-articular injections of high molecular-weight sodium hyaluronan (SH), has been implemented as part of the treatment for OA in humans [7,8], and horses [9].

Polyacrylamide hydrogel [a] (PAAG) is a non-toxic and non-immunogenic biocompatible polymer gel consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide [10,11]. Its biocompatibility in soft tissues (e.g. reconstructive surgery, urology) has been demonstrated [12-14]. Also, PAAG is a non-particulate homogenous gel similar to SH gel in overall structure and tissue compatibility [13], but with a longer-lasting viscous effect, as it is non-degradable [10]. This gel has been used for years in the augmentation of connective tissue in human medicine [14,15]. Experimental studies supported by histopathological observations have shown that PAAG exerts its effect via integration over time within the soft tissues, through a combination of vessel in-growth and molecular water exchange [10,13]. A recent clinical study investigated the effect of PAAG on improving clinical signs of equine OA within the metacarpo(tarso)phalangeal joint or one of the carpal joints (antebrachiocarpal, middle carpal or carpometacarpal) [16]. Thirty-three horses, older than two years with OA located within only one joint were treated intra-articularly with PAAG. At 1, 3, and 6 months after treatment, 81%, 88% and 87% respectively of horses showed improvement in lameness grade compared with baseline. At 6 months, approximately 70% of horses were non-lame [16]. At 12 months, 81% of the horses from the same study population were non-lame [17]. A recent pilot study using an experimental OA model in goats has shown that PAAG was integrated into the synovial membranes of the injected joint, and significantly improved the lameness caused by OA, with 75% (3 out of 4) of the cases becoming non-lame at 4 months post treatment evaluation [18]. A comparative prospective study has demonstrated that horses with OA treated with PAAG significantly improved their clinical signs when compared to horses with OA treated with triamcinolone acetonide combined with SH [19]. Another report has shown that PAAG effectively relieved lameness in horses with distal interphalangeal joint OA [20].

The purpose of this two year prospective clinical study was to investigate the efficacy and duration of action of PAAG for improving clinical signs of OA in the equine metacarpo(tarso)phalangeal or one of the carpal joints. Our hypothesis was that lameness scores would significantly improve and the effect will last at least 2 years in osteoarthritic joints after treatment with PAAG.

Methods

The clinical study was conducted between October 2010 and February 2014 at 5 major equine hospitals (3 in Denmark, 2 in Germany). The study was approved by the National Council for Animal Experimentation (Authorization number: 2010/561-1890). All horse owners gave written informed consent. Client-owned horses older than 2 years with OA confirmed clinically within a single joint (metacarpo(tarso)phalangeal joint or one of the carpal joints (antebrachiocarpal, middle carpal or carpometacarpal)) were included in this clinical study. The confirmation of OA was based on clinical evaluation, lameness abolished after intra-articular anesthesia (10 ml of local anesthetic per joint, horses reexamined in 10 min) and imaging (radiography). Lame horses with severe radiographic abnormalities were also included in the study. Exclusion criteria in this study were horses with lameness problems localized in more than one joint, horses with OA secondary to joint infection, horses that had undergone surgery of this joint (including arthroscopy) within three months preceding the study, and horses with any other anti-arthritic treatment (e.g. nonsteroidal antiinflammatory drugs, corticosteroids, SH) administered to the affected joint within two months preceding the study. Other exclusion criteria included horses that had received any additional anti-arthritic treatment, or undergone surgery during the study period.

The study was designed as a prospective clinical study. This study incorporated horses described in previous reports [16,17]. At baseline (day 0), horses were injected with 2 ml of PAAG into the affected joint. In all cases, this injection was performed the same day as the intraarticular anesthesia. After treatment, horses were rested for the first two weeks with only 10 to 15 minutes hand walking exercise per day, then for the subsequent two weeks, all horses were allowed hand walking exercise for 20 to 30 minutes per day or turnout in a small paddock. All horses were clinically assessed under similar circumstances by clinicians (one per center) different from the one who had originally examined and treated the horse, and unaware of the identity of the horse and whether joints were treated or not at 1, 3, 6, 12 and 24 months post-treatment. All horses received only one injection of PAAG during the study.

Efficacy of the treatment was evaluated by lameness examination of the affected joint, including response to flexion tests. Each horse underwent lower limb (interphalangeal and metacarpo(tarso)phalangeal joints) and carpal flexion tests for 1 min for all limbs. Horses were evaluated in hand on a hard surface in straight lines and in circles. Data relating to case details, including type of activity, limb involved, lameness duration (1–6 months, >6 months), previous joint treatment (yes (type, duration); no), and lameness grade was collected at baseline. Lameness grading [21] was performed at baseline, and at 1, 3, 6, 12 and 24 months. Joint effusion grading (0: no distension, 1: mild, 2: moderate, 3: marked and 4: severe) was only visually assessed at baseline and at 1, 3, 6, 12 and 24 months. The radiographic grading of OA was based on standard radiographic projections [22] for each joint (0: no lesion, 1: mild, 2: moderate and 3: marked) at baseline only. The radiographic grading system used was described previously [23] and was used by a clinician experienced in radiography. The owner's assessment of the result of the treatment (1: not satisfied, 2: slightly satisfied, 3: satisfied and 4: very satisfied) was recorded at 1, 3, 6, 12 and 24 months. Safety of the joint treatment was evaluated through recording of any adverse reaction following joint injection. If the horse was non-lame one month after post treatment, then the horse was allowed to progressively resume its normal activity.

Statistical analysis

The statistical variables used in this study are described in Table 1. Variables potentially associated with the outcome measure "lameness grading" were analyzed using a generalized linear mixed model for ordinal regression with horse identification specified as a random effect. The initial model consisting of all main effects was reduced by backward model selection sequentially removing non-significant effects on a 5% significance level. To investigate the development of joint effusion a similar analysis was done using "effusion grading" as outcome. This analysis was done without including "lameness grading" as explanatory variable. The statistical analysis was done using SAS V9.4.

Results

A total of 43 horses met the inclusion criteria for this study. Only 41, 26 and 40 horses were examined at months 1, 3 and 24 respectively. Table 2 summarizes the descriptive data of the study population. At baseline, the proportion of horses with a moderate to marked radiographic grade was 47%, whereas 53% of horses had mild radiographic grade.

Lameness changes in relation to baseline lameness score. Before treatment (baseline), the proportion of horses with lameness grade 1, 2, 3 and 4 were 26%, 32%, 35% and 7% respectively. In horses with baseline lameness grade 1 (n = 11), 73% were non-lame at 1 month, and 60 to 82% were non-lame at the following controls (3, 6, 12 and 24 months). In these horses, 1 out of 6, 2 out of 11 and 3 out of 10 showed a worsening in lameness grade at 3, 12 and 24 months respectively, after a previous lameness improvement. In horses with baseline lameness grade 2 (n = 14), 62% were non-lame at 1 month, and 79 to 100% were non-lame at the following controls (3, 6, 12 and 24 months). In horses with baseline lameness grade 3 (n = 15), 50% and 62% were non-lame at 1 and 3 months respectively, whereas 80 to 87% were non-lame at the following controls (6, 12 and 24 months). In horses with baseline lameness grade 4 (n = 3), at 1, 3 and 6 months, only 1 horse was non-lame and 2 other horses were non-lame at 12 and 24 months. No lameness worsening was observed in horses with baseline grade 2, 3 and 4.

There was a significant increase in the proportion of non-lame horses between baseline and 1 month, followed by a steady increase between 3 and 6 months, then a stabilization in the proportion of non-lame horses between 6 and 24 months (Figure 1). Concerning the outcome, at 1, 3, 6, 12 and 24 months follow-up, irrespective of the baseline lameness grade, 59%, 69%, 79%, 81/% and 82.5% of horses were non-lame respectively. Figure 2 shows the distribution of the change in lameness grades for the individual horses over the observed time periods. The largest reduction in lameness took place between baseline and 1 month follow-up. After 1,

Table 1 Variables included in the statistical analysis of the study population of 43 horses with osteoarthritis of a metacarpo(tarso)phalangeal joint or carpal joint treated by intra-articular administration of a polyacrylamide hydrogel

Variables	
Horse	43
- Sex	Female, male, gelding
- Breed	Warmblood, racing breed, others
- Type of activity	Dressage, jumping, racing, others
- Time point (months)	0, 1, 3, 6, 12, 24
- Joint	Metacarpo(tarso)phalangeal, carpus
- Limb involved	Front, Hind
- Lameness duration before treatment (months)	1 to 6, > 6
- Previous treatment	Yes, No, Unknown
- Radiographic grading of OA	0 to 3
- Joint effusion grading	0 to 4
- Lameness grading	0 to 5

OA: osteoarthritis.

Table 2 Description of the study population of 43 horses with osteoarthritis of a metacarpo(tarso)phalangeal joint or carpal joint treated by intra-articular administration of a polyacrylamide hydrogel and variables evaluated during a 24 months follow-up

Variable	Data
Horses (no.)	43
Mean (range) age (years)	9.4 (2–15)
Breeds (no. (%))	
Warmbloods	30 (70%)
Racing breeds	8 (19%)
Others	5 (11%)
Horse activity (no. (%))	
Dressage	15 (35%)
Jumping	13 (30%)
Racing	8 (19%)
Other	7 (16%)
Limb involved (no. (%))	
Front	27 (63%)
Hind	16 (27%)
Joint involved (no. (%))	
Metacarpo(metatarso)phalangeal	40 (93%)
Antebrachiocarpal, Middle carpal, Carpometacarpal	3 (7%)
Lameness duration before treatment: (no. (%))	
<6 months	35 (81%)
>6 months	8 (19%)
Previous anti-osteoarthritic therapy	
Yes	37 (86%)
No	6 (14%)
Lameness grading at baseline (no. (%))	
1	11 (26%)
2	14 (32%)
3	15 (35%)
4	3 (7%)
Joint effusion grading at baseline (no. (%))	
0	3 (7%)
1	10 (23%)
2	18 (42%)
3	7 (16%)
4	5 (12%)
Joint effusion grading at 24 months (no. (%))	
0	31 (77.5%)
1	8 (20%)
2	0 (0%)
3	1 (2.5%)
4	0 (0%)
Radiographic grading of OA at baseline (no. (%))	
1 (mild)	23 (53%)
2 (moderate)	9 (21%)
3 (marked)	11 (26%)
Proportion of non-lame horses at (%)	
1 month	80%
3 months	67%
6 months	79%
12 months	81%
24 months	87.5%
Owner satisfaction at 24 months (%)	
Not satisfied	2.5%
Slightly satisfied	7.5%
Satisfied	75%
Very satisfied	75%

OA: osteoarthritis.

3, 6 and 12 months follow-up, 73%, 73%, 81% and 80% of the horses respectively, retained the lameness grade at the following lameness evaluation.

At baseline, joint effusion grade was 0 (7%), 1 (23%), 2 (42%), 3 (16%) and 4 (12%), whereas at 24 months joint effusion grade was 0 (77.5%), 1 (20%), 2 (0%), 3 (2.5%) and 4 (0%). No adverse effects associated with the treated joints were detected during the study period.

At 24 months, 90% of the owners were satisfied or highly satisfied with the outcome of the treatment, whereas 10% of the owners were slightly satisfied or not satisfied with the outcome of the treatment.

The statistical analysis showed a highly significant reduction of the lameness grade (all grades) after baseline ($P < 0.0001$), and a highly significant association between lameness grade and joint effusion ($P < 0.0001$). Estimates for the odds ratios (OR) showed that OR for lower lameness from month 1 to month 24 relative to baseline increased from 20 (95% CI = 6–67) to 58 (95% CI = 12–275) (Table 3). These confidence intervals for OR were wide; however, they were clearly bounded away from 1, which was also reflected by the p-value. Thus, there was a highly significant effect. OR for lower lameness grade was 3.1 (95% CI = 2.0–4.9) when joint effusion decreased by one grade (Table 3).

Estimates for OR showed that joint effusion score decreased significantly over time ($P < 0.0001$), and decreased significantly with radiography scoring ($P = 0.0041$) (Table 4): OR for lower joint effusion grade from month 1 to month 24 relative to baseline increased from 13 (95% CI = 5–34)

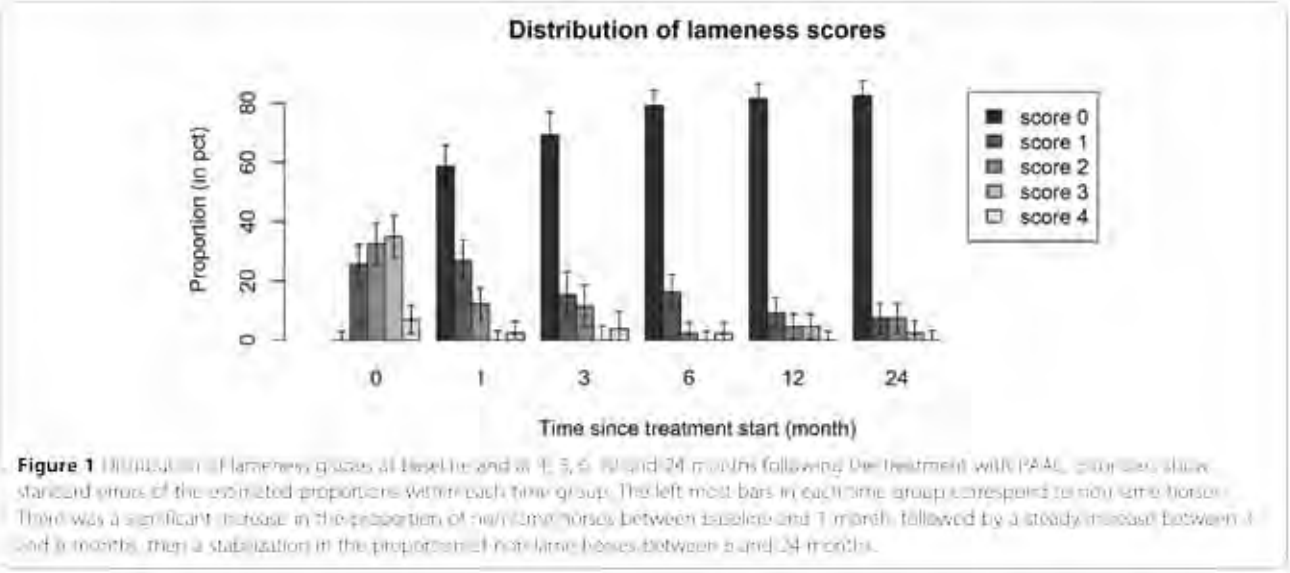

Figure 1 Distribution of lameness grades at baseline and at 1, 3, 6, 12 and 24 months following the treatment with PAAG. Error bars show standard errors of the estimated proportions within each time group. The left most bars in each time group correspond to non-lame horses. There was a significant increase in the proportion of non-lame horses between baseline and 1 month, followed by a steady increase between 1 and 6 months, then a stabilization in the proportion of non-lame horses between 6 and 24 months.

to 172 (95% CI = 46–637). These confidence intervals for OR were wide; however, they were clearly bounded away from 1, which was also reflected by the p-value. OR for lower joint effusion scoring was 3.1 (95% CI = 1.9–5.1) when radiographic grade at baseline was low by one grade.

Discussion

This 2 year clinical study demonstrated that PAAG significantly alleviated lameness in osteoarthritic joints, as assessed by clinical lameness evaluation. A similar outcome was found in a recent pilot randomized controlled study on an experimental OA model in goats, where 75% (3 out of 4) of goats treated with PAAG were non-lame 4 months after the treatment [18]. No adverse effects were observed during the study period in the treated joints, which is consistent with previous studies using PAAG intra-articularly to treat equine OA [16,17,19,20]. PAAG has also proven to be safe in humans for more than 15 years of use [12-14].

The statistical analysis showed a highly significant ($P < 0.0001$) reduction of the lameness grade after baseline. The estimated OR showed an increased reduction over time from OR = 20 from baseline to month 1 to OR = 58 from baseline to month 24. The largest reduction in lameness grade appeared from baseline to month 1. After month 1 the lameness grade continued to decrease, although the difference between months 1, 3, 6, 12 and 24 was non-significant ($p = 0.18$). In particular, the OR was very constant from months 6 to 24. Thus, the clinical improvement in lameness grade was already present one month after PAAG treatment. This suggests that the effect of PAAG on OA might occur mainly

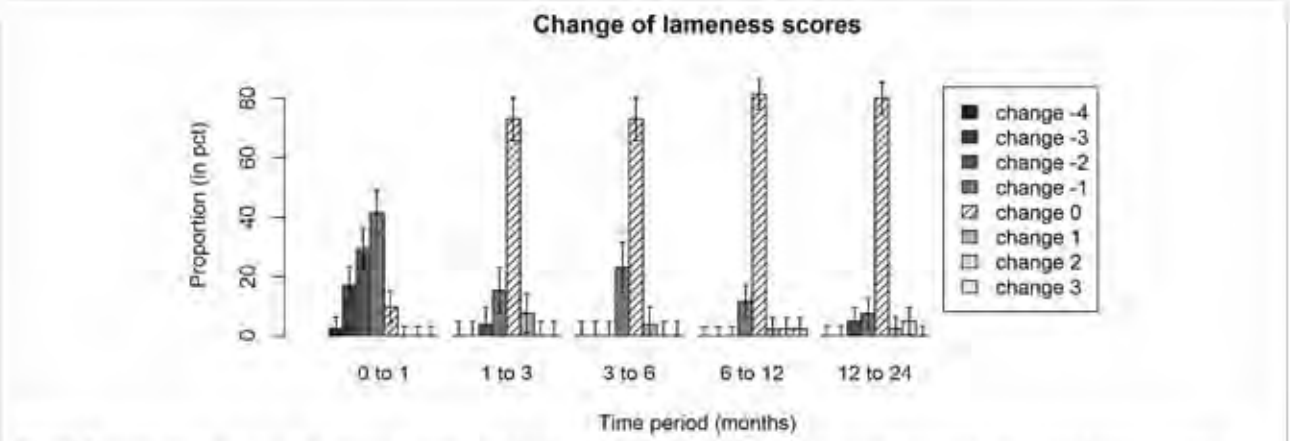

Figure 2 Distribution of change of lameness grades of individual horses over consecutive time points. Error bars show standard errors of the estimated proportions within each time period. The middle bars with shading lines corresponding to horses that have retained their lameness grade over the designated time period. The largest reduction in lameness took place at 1 month follow-up.

Table 3 Estimated odds ratios for lower lameness grade between time points and differences in joint effusion and associated 95% confidence intervals

Odds ratio	Estimate	95% confidence limits	
Month 1 vs Baseline	20.45	6.24	66.98
Month 3 vs Baseline	23.16	5.65	94.85
Month 6 vs Baseline	50.94	12.29	211.05
Month 12 vs Baseline	86.68	15.46	487.14
Month 24 vs Baseline	57.71	12.12	274.71
Effusion −1	4.12	1.87	9.04

Odds ratios for lower lameness from month 1 to month 24 relative to baseline increased from 20 (95% CI = 6–67) to 58 (95% CI = 12–275).

during the first month after treatment and lasts and increases progressively until 6 months, with a stabilization between 6 and 24 months.

Worsening of the lameness grade following a previous lameness improvement was observed in only 3 horses with baseline lameness grade 1 (n = 3/11). Since no radiographic follow-up was performed in our clinical trial, it is difficult to speculate on the reason of lameness worsening in these horses. None of the horses with baseline lameness grade 2, 3 or 4 showed deterioration in lameness.

This clinical study has also demonstrated that joint effusion grade decreased significantly over time ($P < 0.0001$). At baseline, joint effusion was absent in only 7% of the horses, while at 24 months the majority of horses (77.5%) showed no joint effusion of the treated joints. Since lameness grade decreased significantly ($P < 0.0001$) with lower effusion grade, part of the lameness improvement over time can be seen through lowering of the joint effusion.

Although joint effusion was subjectively assessed in this study, PAAG induced a significant decrease in joint effusion in the osteoarthritic joints. However, the mechanism of action of PAAG in reducing joint effusion in osteoarthritic joints needs to be investigated.

Table 4 Estimated odds ratios for lower joint effusion grade between time points and differences in radiography grade at baseline and associated 95% confidence intervals

Odds ratio	Estimate	95% confidence limits	
Month 1 vs Baseline	13.21	3.14	83.96
Month 3 vs Baseline	38.19	11.72	124.40
Month 6 vs Baseline	106.14	32.31	348.61
Month 12 vs Baseline	91.52	28.19	297.07
Month 24 vs Baseline	171.80	46.35	636.76
Radiography grade −1	3.12	1.90	5.13

OR for lower joint effusion grade from month 1 to month 24 relative to baseline increased from 13 (95% CI = 3–34) to 172 (95% CI = 46–637). OR for lower joint effusion grade was 3.1 (95% CI = 1.9–5.1) when radiographic grade at baseline was low by one grade.

The majority of horses (86%) had received a previous unsuccessful anti-osteoarthritic treatment, before receiving PAAG, but there was no correlation between the previous treatment and the outcome lameness variable. In some cases (14%), which were mainly among the last included cases, PAAG was used as a first line treatment based on the encouraging results of the first cases of the study.

At 24 months, 90% of the owners were either satisfied or very satisfied with the outcome of this new OA treatment. This is consistent with the outcome as assessed by the veterinary clinicians (82.5% of non-lame horses at 24 months).

Although conventional concepts of OA emphasize the direct and predominant involvement of cartilage and bone in OA development, it is increasingly recognized that the synovium also contributes to the central pathophysiological event of cartilage matrix depletion. Lack of joint lubrication is postulated to play a significant role in the pathogenesis of OA [24]. This emphasizes the role of viscosupplementation, and hence the improvement of lubrication within the joint, in protecting a joint suffering from OA, and reducing the resulting pain. Recently, a study supported the use of intra-articular lubricin as an adjunct to viscosupplementation for retarding cartilage degeneration and possibly the development of post-traumatic OA [25,26].

Precise characterization of the mechanism-of-action of PAAG on osteoarthritic joints has not yet been established, but histopathological observations on joint tissue from horses [Christensen L, personal communication] and goats [18] have demonstrated that PAAG, like in other soft tissues, becomes integrated within the synovial membrane.

In the goat study [18], the synovial membrane of the joints injected with PAAG had a better elastance when compared to the synovial membrane of the control joints. Osteoarthritic joints typically show joint stiffness which is a major source of pain in OA. This is supported by a recent study on human knee joint stiffness, which showed that the stiffness co-efficient was higher in individuals with painful OA compared to those with normal knees [27].

By integrating the synovial membrane, which may probably decrease the joint capsule stiffness and hence the joint stiffness, PAAG might relieve pain of the osteoarthritic joint. This theory is supported by clinical observations in the study population where osteoarthritic joints that responded well to PAAG were no more painful to passive manipulation of the joints.

The inclusion criteria in the present study were strict in order to maximize the validity of the results. Nevertheless, there were some study limitations including a

low number of horses, the fact that it was a prospective non controlled clinical study, and the subjective assessment of joint distension. A quantitative measurement of joint circumference could have been performed. This was a multi-centre study, which represented another study limitation due to several clinicians involved in the study, and the potential for inconsistency in application of the lameness grading scale among the clinicians and within clinicians at different examinations [28]. In addition, radiography was not used for the follow-up of OA because of its association with a series of concerns including the insensitivity of radiographs to detect early and small changes and the slow progression of OA being a common finding in clinical trials [5]. Repeatability of application of the radiographic grading system was not assessed.

The present study has shown that PAAG relieved or completely removed the symptoms of lameness and the joint distention in osteoarthritic joints and can be considered as a disease-modifying OA therapeutic agent. A recent study on an OA model in goats [18] has shown that PAAG reduces the progression of OA as evaluated by MRI and histopathology, which supports the hypothesis that PAAG contributes to a disease-stabilizing affect. Further work investigating the mechanism of action of PAAG in osteoarthritic joints is required.

Conclusions

PAAG significantly alleviated lameness and joint effusion in osteoarthritic joints in horses. PAAG is a promising, safe and lasting (at least 24 months) new treatment for OA in horses and its further evaluation is warranted.

Endnote

[a] Arthramid® Vet, Contura International A/S, DK-2860 Søborg, Denmark.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AT elaborated the study design. HS, MK, WJ, LC, JR and AT contributed to data collection, or analysis and interpretation. BM carried out the statistical analysis. Writing of the manuscript was carried out by AT and BM (statistical analysis). All authors have read and approved the final version of the manuscript.

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An international multi-centre one year prospective study on the efficacy of an intraarticular polyacrylamide hydrogel in horses with osteoarthritis.

Proceedings ICRS (2012, Turkey)

FACULTY OF HEALTH AND MEDICAL SCIENCES
UNIVERSITY OF COPENHAGEN

An international multi-centre one year prospective study on the efficacy of an intraarticular polyacrylamide hydrogel in horses with osteoarthritis

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Purpose: Osteoarthritis (OA) is a common clinical problem in horses.[1] In OA, elastoviscosity of the synovial fluid is abnormally low,[2] and thus the use of visco-supplementation, for example intra-articular injections of high molecular-weight hyaluronic-acid, has been implemented as part of the treatment for OA in horses.[3] Polyacrylamide hydrogel[a] (PAAG) is a non-toxic and non-immunogenic biocompatible polymer gel consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide.[4] Its biocompatibility in soft tissues has been demonstrated.[5] A recent 6 months clinical trial investigated the effect of PAAG on improving clinical signs of equine OA within the fetlock or carpus.[6] At 6 months, approximately 70% of horses were sound (non-lame).[6] The purpose of this one year prospective study was to investigate the efficacy of PAAG for improving clinical signs of OA in the equine fetlock (metacarpo/metatarso-phalangeal joint) or in one of the carpal joints. Our hypothesis was that lameness scores would improve significantly in OA joints after treatment with PAAG.

Materials & Methods: The clinical trial was conducted between October 2010 and December 2012 at 5 major equine hospitals. Client-owned horses older than 2 years with a confirmed clinical OA within a single joint (fetlock or carpus) based on clinical evaluation, intra-articular anesthesia and imaging (radiography, MRI) were included in this trial. Exclusion criteria in this study were: lameness problems localized in more than one joint, OA secondary to joint infection, horses having undergone surgery of this joint within 3 months preceding the study, and any other anti-arthritic treatment administered to the affected joint within two months preceding the study. Other exclusion criteria included horses that received any additional anti-arthritic treatment, or undergone surgery during the study period. The study was designed as a prospective trial involving horses with symptomatic OA in one joint. At baseline, horses were injected with 2 ml of PAAG into the affected joint, based on a previous study.[6] After treatment, horses were rested for 2 weeks with only 10 to 15 minutes hand walking exercise per day, then for the subsequent 2 weeks, all horses were allowed limited exercise or put-out in a small paddock. All horses were assessed at 1, 3, 6 and 12 months post-treatment where follow-up data were obtained by the same clinician whom performed the original examination. Efficacy of the treatment was evaluated by clinical assessment of lameness in the affected joint, including response to flexion tests. Data relating to case details, including type of activity, limb involved, lameness duration, previous joint treatment, lameness scoring was collected at baseline and at 1, 3, 6 and 12 months. The lameness was graded using the American Association of Equine Practitioners scoring system. Joint effusion scoring was visually assessed at baseline and at 1, 3, 6, and 12 months, as well as radiographic scoring of OA at baseline only. The owner's assessment of the result of the treatment was recorded at 1, 3, 6 and 12 months. Safety assessment of the joint treatment was evaluated through recording of any adverse reaction following joint injection. Statistical analysis: The statistical variables used in this study are described in table 1. Variables potentially associated with the outcome measure "lameness scoring" were analyzed using generalized linear mixed model for logistic regression with the horse as random effect, based on a 5% significance level.

Table 1: The statistical variables included in the study

Variables	Data
- Age (years)	2 to 15
- Sex	Female, male, gelding
- Breed	Warmblood, racing breed, others
- Type of activity	Dressage, jumping, racing, others
- Time point (months)	1, 3, 6, 12
- Leg involved	Front, Hind
- Lameness duration before treatment (months)	1 to 6, > 6
- Lameness scoring	0 to 5
- Joint effusion scoring	0 to 4
- Radiographic scoring of OA	1 to 3

Odds Ratio Estimates

Time	Time	Estimate	DF	95% Confidence Limits	
0	1	0.012	146	0.000	0.043
0	3	0.007	146	0.001	0.039
0	6	0.002	146	<0.001	0.012
0	12	0.002	146	<0.001	0.011
1	3	0.940	146	0.187	1.747
1	6	0.228	146	0.076	0.681
1	12	0.193	146	0.060	0.622
3	6	0.402	146	0.115	1.000
3	12	0.357	146	0.095	1.340
6	12	0.847	146	0.247	2.901

Table 3: Estimated odds ratios and the associated overall p-value.

Variable	Data
Horses (no.)	43
Mean (range) age (years)	9.4 (2-15)
Breeds (no. (%))	
Warmbloods	30 (70%)
Racing breeds	8 (19%)
Others	5 (11%)
Horse activity (no. (%))	
Dressage	15 (35%)
Jumping	13 (30%)
Racing	8 (19%)
Other	7 (16%)
Limb involved (no. (%))	
Front	27 (63%)
Hind	16 (37%)
Joint involved (no. (%))	
Fetlock	40 (93%)
Carpus	3 (7%)
Lameness duration before treatment (no. (%))	
1-6 months	35 (81%)
> 6 months	8 (19%)
Lameness scoring at baseline (no. (%))	
1	12 (28%)
2	13 (30%)
3	15 (35%)
4	3 (7%)
Joint effusion scoring at baseline (no. (%))	
0	3 (7%)
1	10 (23%)
2	18 (42%)
3	7 (16%)
4	5 (12%)
Joint effusion scoring at 12 months (no. (%))	
0	32 (75%)
1	4 (9%)
2	6 (14%)
3	1 (2%)
4	0 (0%)
Radiographic scoring of OA at baseline (no. (%))	
1 (mild)	23 (53%)
2 (moderate)	9 (21%)
3 (marked)	11 (26%)
Proportion of sound horses at (%)	
1 month	66%
3 months	67%
6 months	79%
12 months	81%
Owner satisfaction at 12 months (%)	
Not satisfied	3%
Slightly satisfied	9%
Satisfied	12%
Very satisfied	72%

Table 2: Data of the study population treated with Polyacrylamide hydrogel.

Results: A total of 43 horses met the inclusion criteria for this study. Table 2 summarizes the data of the study population. Thirty seven (86%) horses had previously received another anti-osteoarthritic treatment for the treated joint, and six had no treatment. At baseline, the proportion of horses with moderate to marked radiographic scoring was 47%, whereas 53% of horses demonstrated mild radiographic scoring. At baseline, joint effusion scoring was 0 (7%), 1 (23%), 2 (42%), 3 (16%) and 4 (12%), whereas at 12 months joint effusion scoring was 0 (75%), 1 (9%), 2 (14%), 3 (2%) and 4 (0%). Before treatment, proportion of horses with lameness score 1, 2, 3 and 4 were 28%, 30%, 35% and 7% respectively. Estimated lameness improvement at 1, 3 6 and 12 months were 95%, 88%, 88% and 91% respectively. At 1, 3, 6 and 12 months, 60%, 67%, 79% and 81% of horses were lame free respectively. No side effect was observed in the treated joints. Table 3 demonstrates the estimated odds ratios and the associated overall p-value. There was a significant decrease in lameness score from baseline to time points 1, 3, 6 and 12 months ($p<0.0001$). There was a difference between the 4 time points (1, 3, 6 and 12 months) after the treatment ($F=17.91$, $df = (4,146)$, $p<0.0001$). The estimates for the odds ratios with time=0 in the nominator and time=1, 3, 6 and 12 in the denominator, shows that the effect of the treatment increases over time (OR from 0.012 to 0.002).

Conclusion: This study demonstrated that PAAG significantly alleviated lameness in osteoarthritic joints, as assessed by standard veterinary clinical lameness evaluation, and 81/% of horses were lame free at 12 months. No adverse effects were observed in the treated joints. PAAG is a promising treatment for osteoarthritis in horses, and its further evaluation is warranted.

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[a] Arthramid® Vet, Contura International A/S, Soborg, Denmark.

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Efficacy of a polyacrylamide hydrogel in horses with symptomatic osteoarthritis: an international multi-centre prospective study.

Equine Veterinary Journal - EVJ 44 Suppl. 39 (2012) 2-18

EFFICACY OF A POLYACRYLAMIDE HYDROGEL IN HORSES WITH SYMPTOMATIC OSTEOARTHRITIS: AN INTERNATIONAL MULTI-CENTRE PROSPECTIVE STUDY

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Aims: To investigate the effect of a polyacrylamide hydrogel[1] (PAAG) in improving clinical signs of osteoarthritis (OA) in the fetlock or carpus. PAAG is recently used to intra-articularly treat OA in horses. However, no prospective study on the efficacy of PAAG has been reported.

Methods: Thirty-three horses older than 2 years with a confirmed OA in only one joint (fetlock or carpus) based on clinical evaluation, intra-articular anaesthesia and imaging (radiography, MRI or arthroscopy) have been included in this clinical trial. Horses were injected with 2 ml of PAAG in the affected joint and were followed up at 1, 3 and 6 months. Efficacy was evaluated by clinical assessment of lameness and joint effusion in the affected joint. Safety assessment of the joint was also evaluated. Data relating to case details, type of activity, joint and leg involved, lameness duration, lameness scoring, joint effusion scoring, radiographic scoring and owner assessment were recorded. Factors associated with the outcome measure 'lameness scoring' were analysed using generalised linear mixed model for logistic regression.

Results: Before treatment, the proportion of horses with lameness score 1, 2, 3 and 4 was 27.3%, 33.3%, 33.3% and 6.1%, respectively. The estimated lameness improvement at 1, 3 and 6 months was 81%, 88% and 87%, respectively. At 6 months, approximately 70% (23/33) of horses were lame free. No side effect was observed in the treated joints. There was a significant decrease in lameness score from baseline to 1 month, and from 1 to 3 months, but lameness score was constant from 3 to 6 months.

Conclusions and practical significance: Preliminary results show that PAAG significantly alleviated lameness in OA affected joints, as assessed by standard veterinary lameness evaluation. PAAG is a promising and safe treatment for symptomatic OA in horses, and its further evaluation is warranted.

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HOW DOES LOSS OF DIGITAL SENSORY FEEDBACK AFFECT LOCOMOTION IN THE HORSE?

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Aims: Because horses exhibit qualitatively unchanged locomotion in the absence of distal limb sensation, it is widely assumed that loss of sensory feedback has little or no effect on the natural movement pattern. In contrast, cats require digital sensory feedback for correct foot placement and for the limb to respond appropriately to external stimuli. This suggests that animals with limited distal limb musculature (horses) may not rely on digital sensory feedback during steady-state locomotion. Given these results in cats and the known function of sensory input to reject internally generated neuromuscular noise, we hypothesised that kinematic parameters associated with limb touchdown position in the horse (*Equus caballus*) would show greater variation in the absence of sensory feedback. We predicted that this would result from a reduced ability to reject internally generated neuromuscular noise. Steady state locomotion would therefore be less tightly controlled, resulting in greater variation. To test this, we measured the kinematics of horses with reduced levels of digital sensation.

Methods: Optical motion capture was used to collect kinematic data from 6 horses walking and trotting on a treadmill before and after an abaxial sesamoid nerve block was administered to remove digital sensation in one and then both front limbs.

Results: Contrary to our prediction, preliminary results from 5 horses show that a lack of sensory input results in less variability (Levene's test for homogeneity of variance; trotting; $P<0.001$, $n = 3$ horses) and an increase in duty factor and stance duration for the initially blocked forelimb (linear mixed model $n = 5$ horses, $P<0.001$) for both gaits. We further observed significant changes in the pitch and roll of the body. These results suggest that sensory feedback continuously adjusts foot placement to maintain postural stability.

Conclusions and practical significance: These findings have significant impact for equine clinical practice, in particular in lameness diagnosis and neurectomy for lameness treatment.

A RETROSPECTIVE STUDY OF NOSOCOMIAL INFECTIONS FOLLOWING EMERGENCY EXPLORATORY LAPAROTOMIES

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Aims: To assess prevalence and risk factors of incisional abdominal infections; identify responsible bacterial organisms; describe early clinical follow-up and treatment of these infections; and suggest clinical procedure upon onset.

Methods: A 3 year retrospective study of surgical colics referred to the Lyon Veterinary School, Equine Department was performed. A total of 123 horses were selected among animals surviving beyond one month post operatively. Descriptive data was collected including: nature of secretions, depth of infection, diagnostic methods of the infection, bacterial culture results, treatment protocols. Risk factors were assessed by studying correlations (univariate and multivariate) between infections and preoperative, intraoperative and post operative factors.

Results: Among the 48 cases of incisional infections, 67% were 'deep' (muscular and involving the linea alba) vs. 33% 'superficial' (cutaneous and subcutaneous). Half (50%) of all culture were pure and yielded essentially 4 different species. Isolated bacteria were all resistant to standard post operative antimicrobials and 18% were multiresistant species. We identified 11 factors significantly associated with incisional infections, among which post operative hyperthermia and leucocytosis and post operative peritonitis were found, in the multivariate model, to increase odds of infection, respectively, by 2.9, 10.4 and 9.5.

Conclusions: Despite the lack of accepted standard definition for an incisional infection, our results on prevalence of these infections are consistent with the international literature. Very few factors were found

LOWE et al.

Intra-articular 2.5% polyacrylamide hydrogel (PAAG): A prospective study on 54 thoroughbred racehorses.

39th Bain fallon memorial lectures conference, Annual equine veterinary congress, Posters (2016, Australia)

INTRA-ARTICULAR 2.5% POLYACRYLAMIDE HYDROGEL (PAAG)

for the Treatment of Osteoarthritis in 54 Thoroughbred Racehorses: A Prospective Study

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PURPOSE:

Osteoarthritis (OA) is cited as the most important musculoskeletal disorder in both humans and horses.[1]

Several medications have been evaluated for use in the treatment of horses with OA, including nonsteroidal anti-inflammatory drugs (NSAIDs), polysulphated glycosaminoglycans (PSGAGs), corticosteroids, glucosamine, hyaluronic acid and a combination of the above, along with biotechnological substances such as gene therapy, recombinant or autologous growth factors (platelet rich plasma and autologous conditioned serum) and cells (allogenic and autologous chondrocyte transplantation).[2]

Arthramid® Vet is a non-toxic and non-immunogenic biocompatible polymer gel consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide.[3]

Recent clinical trials have investigated the effect of 2.5% PAAG on improving clinical signs of equine OA in distal interphalangeal (DIP) joints unresponsive to previous treatments, with promising results.[4,5]

Another long term field study evaluated the efficacy of 2.5% PAAG in 43 mostly competition sport horses, with 82.5% of horses being sound (non-lame) at 24 months.[6]

A standardized experimental study to explore the efficacy of 2.5% PAAG in the treatment of induced OA in a goat model also gave encouraging results (3 out of 4 were clinically sound at 4 months).[7] The purpose of this study was to investigate the efficacy of 2.5% PAAG for improving clinical signs of OA in the metacarpophalangeal (MCP) and carpal joints of Thoroughbred racehorses whilst in training using an independent observer at a single facility. Our hypothesis was that lameness scores would improve significantly in OA joints after treatment with 2.5% cross-linked PAAG.

MATERIALS & METHODS:

54 Thoroughbred horses aged 3 to 7 years gave a total of 108 joints with confirmed OA based on clinical examination, intra-articular analgesia and imaging (radiography), were included in the clinical trial.

Exclusion criteria included OA secondary to joint infection, horses having undergone surgery of this joint within 3 months preceding the study, and any other anti-arthritic treatment administered to the affected joint within two months preceding the study. Other exclusion criteria included horses that received any additional anti-arthritic treatment, or underwent surgery during the study period. Horses were injected with 2 ml of 2.5% cross-linked PAAG (Arthramid® Vet) in the affected joint(s) and were followed up at 1 week, 4 weeks, 12 weeks and 24 weeks by a veterinary observer blinded to the treatment administered.

Horses were rested for 48 hours after treatment before returning to normal training. Efficacy was evaluated by clinical assessment of lameness (including flexion) in the affected joint(s). Variables at each examination point relating to case details, limb and joint involved, previous treatments, duration of lameness, lameness scoring (AAEP 0-5 scale), radiographic scoring (0-3), joint effusion scoring (0-3), joint range of motion scoring (0-3), and owner satisfaction (1-4) were recorded under standardized guidelines detailed within Table 1.

Follow up was by re-examination and personal interview. Safety assessment was evaluated through recording any adverse reaction following joint injection.

Statistical analysis: Variables associated with "lameness" were analysed with the horse as random effect, based on a 5% significance level.

CONCLUSION:

This study demonstrated that 2.5% cross-linked PAAG significantly alleviated lameness from OA in carpal and metacarpophalangeal joints in racing Thoroughbreds, as assessed by standard veterinary clinical lameness evaluation. 66.7% of horses (36/54) were lame free at 24 weeks (6 months).

Results show a level of efficacy of PAAG similar to that of other published studies.[6] Horses that respond well appear to do so within the first 4 to 6 weeks of treatment so reassessment at that stage may be important to reconfirm the diagnosis and/or repeat 2.5% PAAG treatment if they are still lame. Further response to treatment after 12 weeks is limited occurring in only one of the reported cases. The response to lameness in predominately carpal joints is promising, as is having a treatment that responds sufficiently long term.

The dramatic and significant reduction in joint effusion seems to support the recent suggestion that 2.5% PAAG may have a disease modifying effect from its incorporation into the synovial membrane and high visco-supplementation properties.[7] No adverse reactions were observed in any of the treated joints. A control group would be preferable and should be the focus for further research. Although animal ethics concerns and owner compliance remain a challenge in clinical trials use of a positive control may be a practicable solution to achieve this desired outcome. In this study some subjects (24%) had already failed to respond to conventional treatments so they may have acted as their own positive controls. This has also been suggested in previous studies.[5]

The only significant difference in the management of each case was the 2.5% cross-linked PAAG treatment as each horse was under the same trainer, and therefore track and environmental conditions and veterinary management.

RESULTS:

54 horses with a total of 108 OA joints met the inclusion criteria.

Table 1 below summarizes the data of the study population. At baseline 43% (46/108) of joints had a radiographic grade of zero, whereas 57% (62/108) had mild, moderate or severe radiographic grades.

Lameness was localized to the intercarpal joint in 85% (92/108) of the joints, the radiocarpal joint in 3% (3/108), and the MCP joint represented 12% (13/108) of the joints being treated.

Before treatment the proportion of horses with lameness score 1 was 15% (8/54), 2 was 42% (23/54) and 3 was 43% (23/54). No horses were included with grade 4 or 5 lameness. Of the total number of horses 24% (13/54) had been non-responsive to conventional joint treatments previously. Estimated lameness improvements were 5.6% (3/54) at 1 week, 48.1% (26/54) at 4 weeks, and 66.7% (36/54) at 12 weeks. One other patient improved after that time so at 24 weeks approximately 68.5% (37/54) of the horses were lame free, with a further 14.8% (8/54) having improved to some degree and enough to remain in race training.

Effusion scores decreased significantly in all horses that had effusion at baseline (day 1). No side effect was observed in any of the treated joints. No significant difference in joint ROM was detected during the study period. Owner satisfaction scores improved overall to a total of 88% (47/54) being either satisfied or very satisfied with the outcome to treatment. Estimated odds ratios demonstrated a significant difference between lameness at 4 weeks ($p<0.01$) and at time points 12 and 24 weeks after treatment ($p<0.001$).

Table 1. **Data of the study population treated with Polyacrylamide hydrogel.**

VARIABLES	DATA	
	No.	*Percentage %*
Thoroughbred Horses (Total No.)	54	
(Female, Male, Gelding)		
Mean (range) age (years)	3-7 yrs	
Total number of joints confirmed with OA	108	
Horse activity: Racing (Total No.)	54	100%
Limb involved		
Front only	108	100%
Joint involved		
Metacarpophalangeal (Fetlock)	13	12%
Intercarpal	92	85%
Radiocarpal	3	3%
Lameness duration before treatment		
1-6 months	53	99%
> 6 months	1	1%
Lameness scoring at baseline *Scoring: 0 to 5*		
1	17	15%
2	45	42%
3	46	43%
4 & 5	0	0%
Joint Effusion scoring at baseline		
0 *(None)*	73	68%
1 *(Mild)*	22	20%
2 *(Moderate)*	13	12%
3 *(Moderate-Severe)*	0	0%
4 *(Severe)*	0	0%
Joint Effusion scoring at 24 weeks		
0 *(None)*	103	96%
1 *(Mild)*	4	4%
2 *(Moderate)*	0	0%
3 *(Moderate-Severe)*	0	0%
4 *(Severe)*	0	0%
Radiographic scoring of OA at baseline		
0 *(None)*	46	43%
1 *(Mild)*	37	34%
2 *(Moderate)*	7	6%
3 *(Severe)*	18	17%
Proportion of sound horses *Scoring: 0 (Full Degrees) To 3 (Zero Degrees)*		
1 week	3	5.6%
4 weeks	26	48.1%
12 weeks	36	66.7%
24 weeks	37	68.5%
Owner satisfaction at 24 weeks		
Very dissatisfied		6%
Slightly satisfied		6%
Satisfied		20%
Very satisfied		68%

REFERENCES: [illegible]

CHAPTER 2

SOFT TISSUES & SYNOVIUM

TNIBAR et al.

Evaluation of a polyacrylamide hydrogel in the treatment of induced osteoarthritis in a goat model: a randomized controlled pilot study.

Proceedings OARSI (2014, Volume 22, Supplement, S1-S490, France)

CHRISTENSEN et al.

Tissue integration of polyacrylamide hydrogel: an experimental study of periurethral, perivesical and mammary gland tissue in the pig.

Dermatology Surgery (2008) 34:S68-S77

CHRISTENSEN et al.

The effects of polyacrylamide hydrogel in normal and osteoarthritic animal joints.

Osteoarthritis Research Society International (OARSI), World annual congress, Posters (2016, The Netherlands)

TNIBAR et al.

Evaluation of a polyacrylamide hydrogel in the treatment of induced osteoarthritis in a goat model: a randomized controlled pilot study.

Proceedings OARSI (2014, Volume 22, Supplement, S1-S490, France)

FACULTY OF HEALTH AND MEDICAL SCIENCES
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EVALUATION OF A POLYACRYLAMIDE HYDROGEL IN THE TREATMENT OF INDUCED OSTEOARTHRITIS IN A GOAT MODEL: A RANDOMIZED CONTROLLED PILOT STUDY

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Purpose: Polyacrylamide hydrogel[a] (PAAG) is an inert, non-degradable, non-immunogenic polymer gel with high viscoelasticity consisting of 97.5% sterile water and 2.5% cross-linked polyacrylamide. Its biocompatibility in soft tissues has been demonstrated[1]. PAAG has recently been tested for the treatment of osteoarthritis (OA) in horses with highly encouraging results[2-4], however no standardized experimental studies have been done to explore its efficacy. The purpose of this study was to evaluate PAAG in the treatment of induced OA in a goat model.

Methods: A randomized controlled study was conducted involving goats with induced OA on the left stifle (knee) joint. OA was surgically induced by the transection of the medial collateral ligament, the bisection of the medial meniscus at its midpoint and partial-thickness incisions of the cartilage of the medial tibial plateau. Goats were allowed free exercise, and 3 months after surgery they were randomly divided into 2 groups: group 1 (n=4): PAAG and group 2 (n=2): saline solution (control). Treatments were injected intraarticularly. MRI of the left knee had been performed prior to surgery, at the time of injection (3 months) and 4, 5 and 7 months post-surgery. T1, T2/PD and Stir weighted MRI images were used to assess OA. All goats were clinically evaluated on ground and on treadmill and videotaped for evaluation by 3 blinded observers. Haematology, biochemistry and acute phase proteins were also assessed. The goats were euthanized 7 months after surgery, and gross pathology and histopathology, including immunohistochemistry for nerve endings (n=3 joints), were performed on both femorotibial joints. The hardness of the joint capsule was measured in both groups using Instron ® 5564 testing system (HIS GlobalSpec, MA, USA).

Fig. 1: Goat undergoing a treadmill examination for lameness assessment.

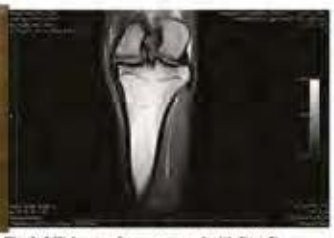

Fig. 2: MR image of a goat treated with PAAG showing osteoarthritis changes at the medial femorotibial joint.

Fig. 3: The hardness of the joint capsule is measured using an Instron testing system.

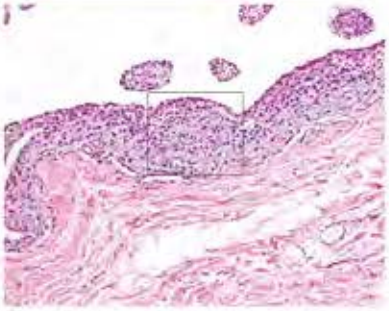

Fig. 4: Histopathology of the synovial membrane of a goat's knee 4 months after treatment with PAAG (HE 10x).

Results: At the end of the study, 3 out of 4 of the goats treated with PAAG were clinically sound, and 1 of them had not improved, whereas the 2 control goats were still lame. In both groups, the values of haematology, biochemistry, or acute phase proteins were within normal range. MRI showed that in group one, 3 out of 4 goats had a decrease followed by a stabilization of OA lesions, while 1 goat had a mild progression of the OA lesions. In group 2, both goats had a mild or marked increase of OA lesions. Gross pathology inspection in group 1 demonstrated that all the operated knees showed typical signs of OA. The inner synovial lining was thickened, and the cartilage surface was uneven in all cases. The gel was seen in various amounts adhering to the inner side of the joint capsule in all the goats of group 1. Gross inspection of both goats in group 2 also showed cartilage lesions and synovial thickening, but the histopathological investigations revealed this to be more prominent in group 1 than in group 2. It comprised angiogenesis, collagen and synovial cell increase, and in the injected goats, also the gel. The nerve endings were normal looking and in normal numbers. The investigation of the joint capsule hardness showed that in the treated knee of the goats of group 1, the medial side (injected with PAAG) was always less hard than the lateral side.

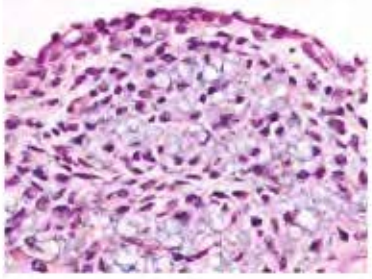

Fig. 5. Magnification area as in Fig.4. This shows angiogenesis, collagen, synovial cell increase and PAAG gel (blue vacuoles) integrating the synovial membrane (HE 40x).

Conclusions: This study demonstrated the efficacy of a novel treatment of OA, with 3 out of 4 of the goats treated with PAAG being clinically sound. Treatment with PAAG did not have any influence on haematology, biochemistry, or acute phase proteins. It induced a moderate synovial hyperplasia of the inner side of the capsule with trapped (integrated) gel, increased angiogenesis and collagen production. Preliminary pathology and joint capsule hardness data suggest that PAAG might act mainly on the joint soft tissue and especially the synovial membrane. PAAG might have 2 effects on OA joints: 1- Joint capsule was less hard on the treated (medial) than on the non-treated (lateral) side and had a lower hardness when compared to group 2. OA joints typically show joint stiffness - a major source of pain in OA. By decreasing the joint capsule hardness, and thus joint stiffness, PAAG might relieve the pain in the OA joint ("disease-modifying" effect). 2- MRI and pathology investigations have revealed a stabilization of OA lesions in the goats of group 1, which might be explained by the mechanical effect through the high viscosupplementation provided by PAAG that was still present in the joint cavity ("disease-stabilizing" effect). No adverse reaction was seen following intraarticular injection of PAAG. More investigations are needed to fully understand the mechanism of action of PAAG in improving clinical signs and in stabilizing OA. This pilot study may be used as a basis for further studies using larger animal numbers.

[a] **Arthramid® Vet, Contura International A/S, 2860, Soborg, Denmark.**

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CHRISTENSEN et al.

Tissue integration of polyacrylamide hydrogel: an experimental study ***of periurethral, perivesical and mammary gland tissue in the pig.***

Dermatology Surgery (2008) 34:S68-S77

Tissue Integration of Polyacrylamide Hydrogel: An Experimental Study of Periurethral, Perivesical, and Mammary Gland Tissue in the Pig

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BACKGROUND Polyacrylamide hydrogel (PAAG) is a nondegradable water-based polymer with high viscoelasticity. The gel is used as a tissue filler, the only risk being prolonged infection with anaerobic, contaminating microorganisms if not treated early with broad-spectrum antibiotics.

OBJECTIVE With silicone gel as reference, PAAG tissue integration and migration was studied in a longitudinal study of the pig.

MATERIALS AND METHODS Forty-one pigs were used. PAAG and silicone gel were injected into mammary tissue, and PAAG was injected into urethral or bladder wall or the anal canal. Tissues and regional lymph nodes were examined at 1, 1 1/2, 3, 3 1/2, 6, 12, and 14 months, and other lymph nodes and organs were examined at 1, 6, 12, and 14 months.

RESULTS PAAG was invaded by macrophages and giant cells that were gradually replaced by a network of fibrous tissue. Silicone gel was seen inside these cells or as large vacuoles, surrounded by a fibrous capsule. Regional lymph nodes contained PAAG only at 1 1/2 months and silicone gel at 12 months.

CONCLUSION PAAG is a stable, viscoelastic bulking agent, which unlike silicone gel is slowly integrated within its host tissue via a thin fibrous network. Long-term risk of fibrosis and migration is minimal.

This study was financially supported by Contura A/S, Soborg, Denmark.

Injectable bulking agents have been used for more than 100 years in the treatment of wrinkles, scars, defects, reflux, and incontinence,[1–4] but problems with biodegradation, biocompatibility, fibrosis, infection, and migration have prevented their success. Polyacrylamide hydrogel (PAAG) is an atoxic and nonimmunogenic polymer gel consisting of 2.5% to 3.5% cross-linked polyacrylamide and 96.5% to 97.5% water.[5–12] It is resistant to degradation and has a widespread use in ophthalmic surgery, drug treatment, food packaging, and water purification.[5–9,13] PAAG has been used in plastic surgery for aesthetic purposes in the former Soviet Union and China for the past 15 to 20 years,[8–11,14–16] and in Europe for the past 7.[17–21] We have previously shown that the gel stays in human breast tissue for at least 8 1/2 years after the injection and is accompanied by a modest or no tissue reaction without capsular fibrosis or calcification.[10] We have also observed that PAAG injected into the subcutaneous tissue of the human face initially elicits a foreign-body reaction, which disappears with time leaving the host tissue inert to the gel at 3 years.[19] A recently published study in rabbits has shown that the bulking effect is preserved, at least for 7 months.[22] This prospective experimental study on minipigs and normal Danish pigs was undertaken with the sole purpose of testing the histologic effect on host tissue of the hydrophilic PAAG, as opposed to the hydrophobic polymer gel, silicone gel, over time,

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and to study the extent of gel migration, both locally and along the reticuloendothelial system (RES). The gel was injected subcutaneously, submucosally or intramuscularly, and all injected tissue, as well as regional and distant lymph nodes and RES organs, were examined macro and microscopically at 1, 1 1/2, 3, 3 1/2, 6, 12, and 14 months.

Materials and Methods

A total of 41 pigs, 11 normal Danish pigs and 30 Göttingen SPF minipigs, all 3 to 4 months of age, were used. Twelve of the minipigs were injected with low-viscosity (LV) PAAG (Aquamid, Contura A/S, Søbory, Denmark), comprising 2.5% polyacrylamide and 97.5% water, and 12 were injected with a specially produced intermediate-viscosity (IV) PAAG comprising 3.1% polyacrylamide and 96.9% water (R. Smith, personal communication, Chempilots A/S, Farum, Denmark, 2006). Another 6 minipigs were injected with silicone gel obtained from silicone breast prostheses (Allergan, Inc., Irvine, CA; style 20 smooth round silicone). The viscosity of this gel corresponded to that of the IIV PAAG.

The normal-size pigs were injected intravesically with the IV and a high-viscosity (HV) PAAG, comprising 3.1% and 3.4% polyacrylamide, respectively (R. Smith, Chempilots A/S) and intraurethrally with the LV PAAG (Aquamid; $n = 6$). An additional five pigs were injected perianally with the IV PAAG ($n = 5$).

All minipigs received six deposits (three on each side) of each 5-mL bolus. Five of the normal Danish pigs received two deposits of each 1 mL perianally, and an additional six received two 1-mL deposits into the bladder wall and two 0.5-mL deposits into their urethral wall. However, because the urethral wall of the pig is very thin—one-third of the size in man—it was noticed that a large part of the gel reached a final positioning along the serosal surface of the urethra—within the pelvic cavity.

The pigs were observed daily for deviations in eating and drinking behavior and physical activity. Blood tests were taken just prior to the injection and during the last week before termination. These include hemoglobin, red cell count, hematocrit, mean cell volume, mean cell hemoglobin concentration, white cell count, differential leukocyte count, platelet count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, γ-glutamyltransferase, cholesterol, carbamide, creatinine, glucose, sodium, potassium, calcium, protein, protein electrophoresis, and globulin.

The pigs were euthanized—two or three at a time after 1, 1 1/2, 3 1/2, 4, 6, 12, and 14 months. A macroscopic examination of all injection sites was done by naked eye inspection and palpation (breast deposits) or after dissection (urethra, bladder, and anal canal). Regional lymph nodes were isolated, and after opening the cranial, thoracic, and abdominal cavities, all organs were examined in situ. Microscopic examination using H&E and van Gieson/Alcian blue morphology staining was carried out on all tissues. Pigs euthanized at 1, 3, 4, 6, 12, and 14 months also had lymph nodes examined from the neck, the mediastinum, paraaortically, and the portal area, and pigs euthanized at 6, 12, and 14 months also had random samples, one to five (mean, three), examined from the thymus, lungs, stomach, liver, spleen, kidneys, and nerve system (sciatic nerve or medulla oblongata or brain). Microscopic examination of the bone marrow was made at 6 and 14 months.

The study on minipigs was carried out at the Scantox Laboratories (Lille Skensved, Denmark) in accordance with the OECD principles of good laboratory practice (GLP). The study on normal Danish pigs was carried out at Skejby University Hospital, Denmark, and had been approved by the local ethics committee (No. 1999-1998-561-64).

Results

Clinically, no deviations from normal were observed in regard to behavior, body weights and blood

values, and no treatment-related adverse reactions were recorded. Macroscopically, the PAAG deposits differed significantly from the silicone gel deposits. At 1 month, the large PAAG deposits placed submucosally appeared to have spread out into numerous minor deposits. The same was found at 3, 6, and 12 months with no apparent reduction in bulk size over time. A thin fibrous capsule was noticed in a few of the deposits of HV gel. Silicone gel was seen as one large or a few smaller firm masses of clear material, always surrounded by a 1- to 2-mm-thick fibrous capsule.

Gross inspection of the vesicourethral specimens confirmed that PAAG had been injected into the urethral/bladder wall, both intramurally and into the pelvic cavity, which contained large collections. Submucosal bulges of different sizes corresponding to the gel deposits were seen in all cases (Figure 1). The variation in size occurred at random among the different specimens and from one side to the other of the same specimen. Bleeding was seen in a few cases but there were no signs of hardening or infection.

A total of 254 regional lymph nodes were isolated (52 at 1 and 1 1/2 months; 64 at 3, 3 1/2, and 4 months; 84 at 6 months; and 35 at 12 and 18 at 14 months). They showed variation in size, depending on site and, for regional nodes, time since injection. In PAAG-injected pigs regional lymph nodes were of the same mean size at 1, 1 1/2, 9, 12, and 14 months, but at 3, 3 1/2, 4, and 6 months they were generally enlarged. In silicone gel–injected pigs the regional lymph nodes ($n = 56$) examined at 1 and 12 months were similar to those injected with PAAG at the same times.

All other lymph nodes ($n = 281$ from PAAG injected pigs and 81 from silicone injected pigs) were comparable in consistency and size at all times. All internal organs were normal as well.

On histologic examination, the PAAG looked in all sections like a homogenous mass with numerous empty holes and a tendency to retract itself from boundaries (Figures 1B, 2B–2D, 4, and 5). Silicone

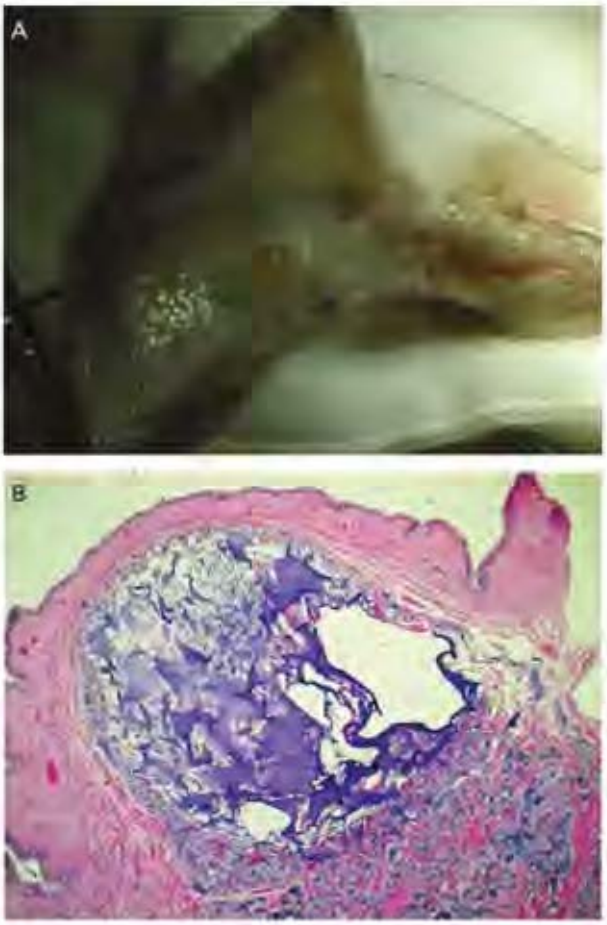

Figure 1. Bladder mucosa containing a large bulge of polyacrylamide gel: (A) macro photo; (B) micro photo (H&E, ×20). The gel is invaded by connective tissue, predominantly toward host tissue not covered by an epithelial lining.

gel appeared in most cases as just a thin, irregular refractile rim along the deposits or vacuoles (Figure 3). The dehydration process, which the tissue section is subjected to during routine processing for histologic examination, was responsible for the PAAG retraction, and the fat extraction process, also part of routine processing, was responsible for the silicone gel loss. Once injected, the PAAG tended to disperse in smaller deposits, lateralizing along natural boundaries (e.g., fascia). This was not seen for the silicone gel. Deposits of the HV PAAG tended to show a more rounded appearance and a slightly darker color than the LV and IV gel types.

Histiocytes (macrophages and foreign-body giant cells) were present in both PAAG and silicone gel

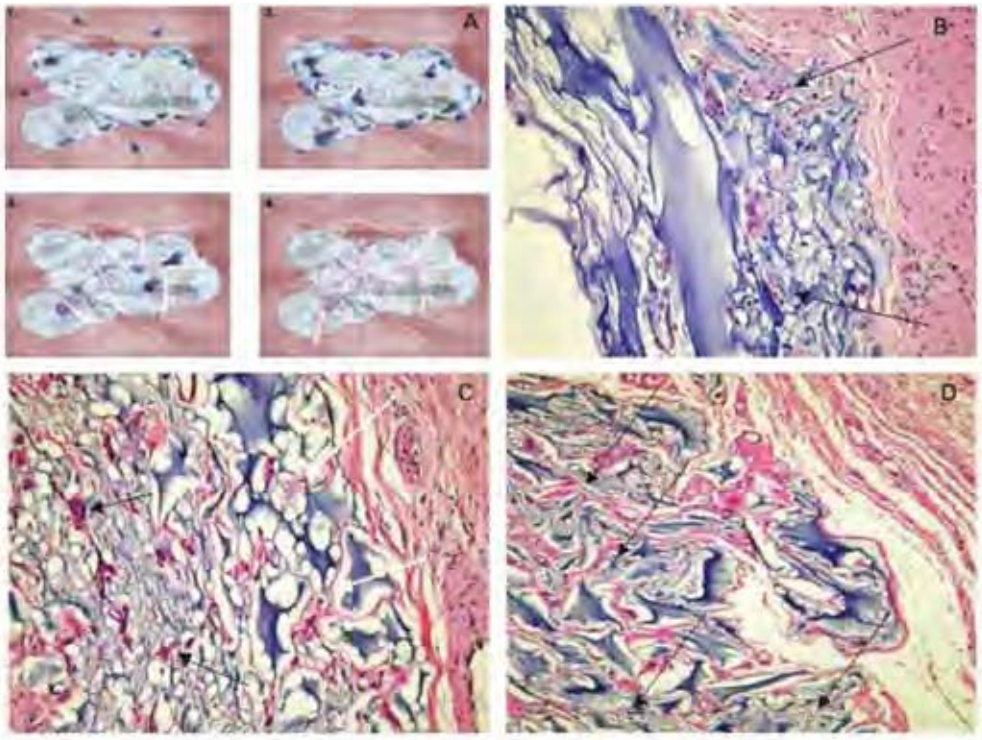

Figure 2. Polyacrylamide hydrogel—host-tissue interaction. (A) A schematic drawing showing the four steps in PAAG tissue integration. Macrophages congregate along the periphery of the gel (1), they start to invade the gel (2), fibrous strands are emerging (3), and a complete fibrous network has formed (4). (B–D) Histology of PAAG–tissue interaction (H&E, × 40). In the beginning most macrophages and giant cells appear at the outer rim of the gel deposit, adjacent to the host tissue (B, arrows). Later, most macrophages and giant cells form a network within the deeper part of the gel deposit (C, black arrows), whereas the outer rim is traversed by a network of connective tissue fibers (C, white arrows). At 14 months, most of the gel deposit is traversed by a network of connective tissue fibers (D, arrows).

deposits. However, whereas these cells had engulfed the silicone gel (Figures 3C and 3D) or surrounded the gel (Figure 3), they had entered the PAAG and were gradually replaced by a fibrous network, the progress of which depended on the size of the gel deposit and its relation to a covering surface (Figures 1B and 2).

With the exception of one area with a small calcification within the bladder epithelium, the covering surface (skin, mucosa) was intact and normal looking. Neither PAAG nor silicone gel deposits showed any signs of fragmentation, calcification, necrosis, or infection.

Passive Migration

At sites where large PAAG collections had been observed lying freely in the pelvic cavity, the adjacent fat showed a more pronounced host reaction (Figure 4). A 50-μm broad rim, consisting of an irregular mixture of macrophages, foreign-body giant cells, and gel was seen at 1 1/2 months (Figures 4A and 4B). A similar but only 30-μm rim was seen at 3 1/2 months (Figure 4C), and at 14 months only a thin rim, consisting of mainly connective tissue, remained (Figure 4D).

Regional lymph nodes and a few neck lymph nodes showed evidence of forced migration (high injection pressure) or passive drainage from large PAAG masses injected erroneously into the pelvic cavity. Sixty percent of local lymph nodes from the breast region and 40% of local lymph nodes and one section from the spleen capsule from the perivesical region showed gel within their lymph vessels. This was seen for PAAG (Figure 5) as well as for silicone gel (Figure 6). Local lymph nodes and vessels from the anal region with only small intramural deposits contained no gel.

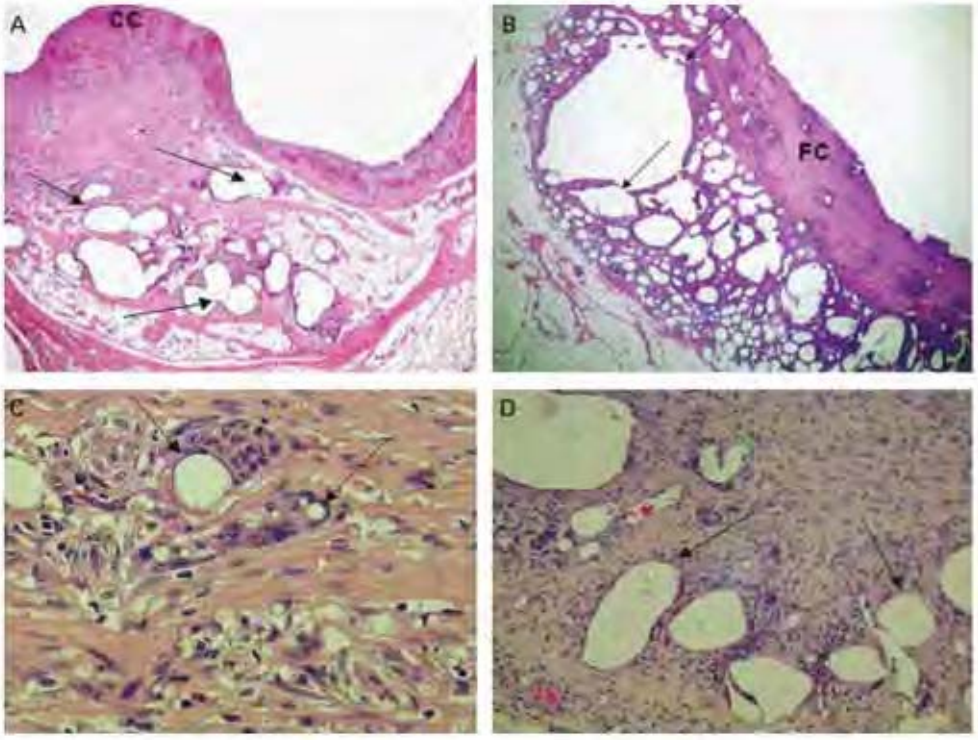

Figure 3. (A) Silicone gel deposit at 1 month consisting of vacuoles (arrows) surrounded by histiocytes and a cellular capsule (CC). (B) At 12 months the capsule is more fibrous (FC). At both 1 and 12 months, macrophages and giant cells contain large droplets of silicone (C, arrows), which are coalesced into larger vacuoles (D, arrows), surrounded by these cells (C, H&E, × 40) and (D, H&E, × 60).

Active Migration

Evidence of a transient macrophage-mediated regional migration of PAAG was seen at 1, 1 1/2, 3, and 3 1/2 months with recognizable PAAG at 1 and 1 1/2 months (Figures 7 and 8). The number of cells and the overall lymph nodes size was reduced at 12 and 14 months.

Evidence of a macrophage-mediated migration was also seen for the silicone gel, but only at 12 months. Small silicone droplets appeared within sheets of macrophages, located within the lymph node sinuses (Figure 9). All other lymph nodes looked normal.

Discussion

This experimental study was undertaken with the sole purpose of systematically investigating the histologic and biologic effect of PAAG on soft and muscle tissue. Clinical studies have shown that PAAG is an excellent filler for soft tissue augmentation,[10,11,17,18] just as silicone gel has been used successfully in intradermal applications, e.g., acne scarring.[23] However, we did not have a direct translation of the results into a clinical setting in view but were only interested in how the hydrophilic PAAG as opposed to the hydrophobic silicone gel would interact with surrounding host tissue.

We chose the pig, because it is the experimental animal closest related to man in respect to anatomy and histology, and a reason for using large gel deposits was the higher probability of obtaining and hence detecting regional or distant migration. In accordance with a recent experimental study in rabbits[22] and several clinical studies,[17,18,20,21] we could confirm that PAAG retains its bulking effect for a long time. Optimally, the study should have been carried out for up to 5 or 10 years to test permanency and long-term effects of both gels, but a maximum follow-up of 14 months gave sufficient insight into the

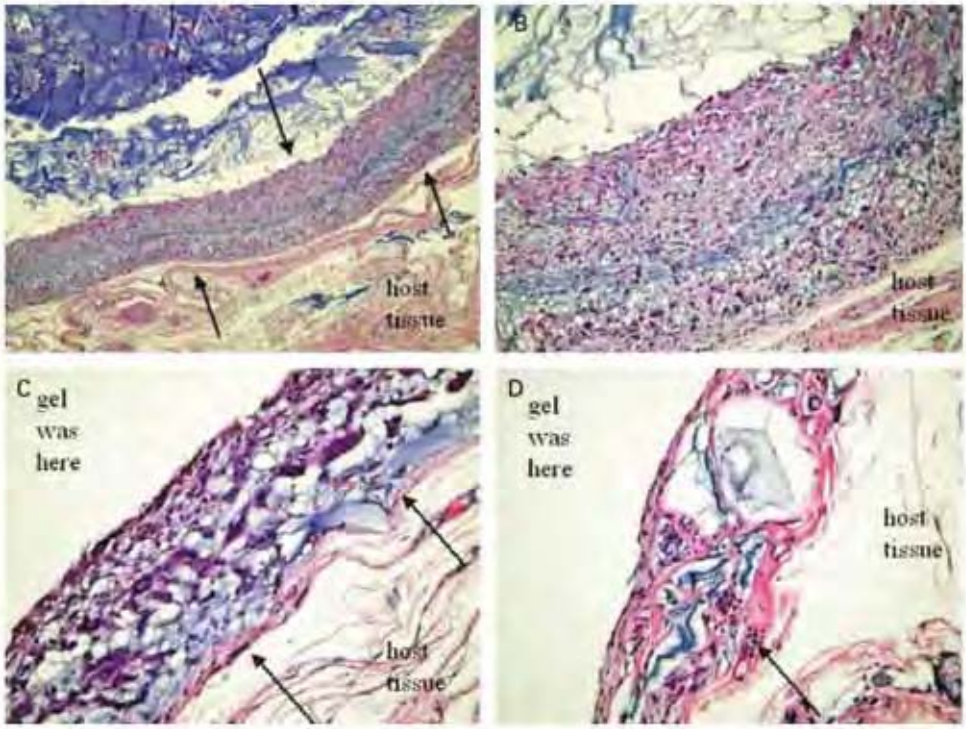

Figure 4. Host reaction to large gel deposits. (A) At 1 1/2 months, a thick rim consisting of macrophages, foreign-body giant cells, and gel has formed around large gel deposits lying in the loose host connective tissue (arrows; H&E, × 20). (B) Close-up view of the cellular rim seen in (A), displaying individual cells (H&E, × 40). (C) At 3 1/2 months, a thinner rim appears around the large gel deposits (arrows). Gel, which has been present centrally in the deposit (top left), has been extracted during histological processing (H&E, × 60). (D) At 14 months, the rim consists mainly of connective tissue and gel. Only a few macrophages and giant cells remain (arrow; H&E, × 40).

integration process for the peripheral borders of the PAAG deposits, and it must be assumed that the integration process continues, as long as untraversed gel remains.

The control gel (silicone gel) also retained expectedly its bulking effect, but as a hydrophobic gel, it was not, like PAAG, entered by macrophages and giant cells. Instead it was seen inside these cells or it

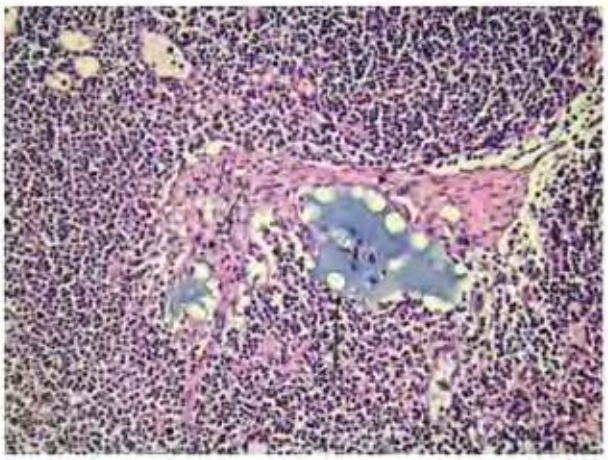

Figure 5. Connective tissue trabeculum of a regional lymph node containing PAAG within a small lymph vessel (arrows; H&E, × 40).

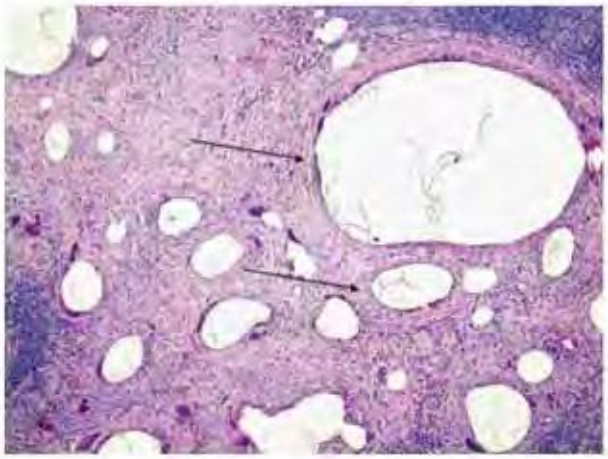

Figure 6. Connective tissue trabeculum of a regional lymph node containing vacuoles of silicone gel (arrows; H&E, × 40).

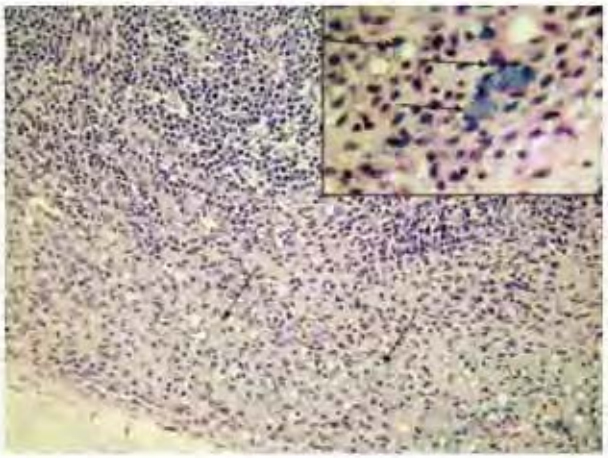

Figure 7. Regional lymph node with a subcapsular collection of macrophages containing PAAG (arrows; H&E, × 40). Inset: close-up of the macrophages (arrows; H&E, × 90).

formed vacuoles surrounded by a firm capsule of fibrous tissue.

Prospective clinical studies of patients being treated with PAAG for aesthetic purposes,[17,18] human immunodeficiency virus lipoatrophy,[20] and stress urinary incontinence[21] have already been published, and more are under way. Clinically, the gel appears to be effective and long-lasting, with no tissue hardening or local dissection/migration complications that have been described for some of the other fillers.[24–26] The advantage of PAAG is its viscoelasticity and high water content, which gives it

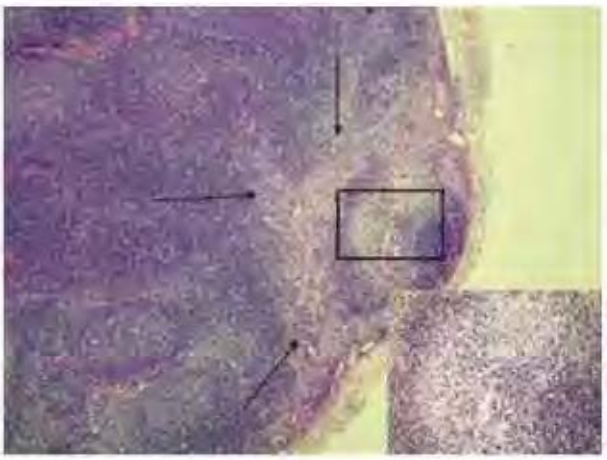

Figure 8. Regional lymph node containing an area of subcapsular histiocytosis (arrows; H&E, × 25). Inset: a close-up of the area (H&E, × 40).

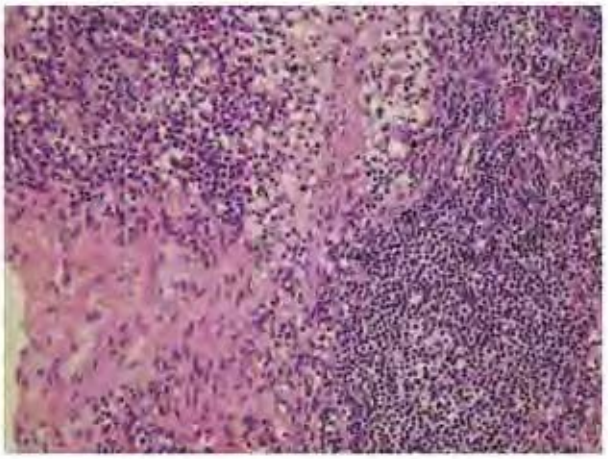

Figure 9. Regional lymph node with macrophages containing a slightly refractile material within their cytoplasm (silicone gel; arrows; H&E, × 60).

a constant molecular interaction with its surroundings. This probably facilitates the in situ anchoring by the fibrous network seen in this study and prevents, in contrast to silicone gel, the formation of a thick fibrous capsule, which gives tissue hardening and calcifications. The only disadvantage of the gel is the infection risk.[19,27] The incidence of infection following injection is the same as for other fillers (approximately 0.1%), but the atoxic PAAG serves as an excellent growth medium for bacteria, and if not treated immediately with broad-spectrum antibiotics in high dosage, the infection can be long-lasting and difficult to treat.[27] On the other hand, the infection risk is always associated with the injection procedure, and late debut of an infection (more than 1 year after the injection) has not been described.[27] Once the gel has been integrated within the tissue and a network has formed with scavenger cells close at hand, a de novo infection is unlikely to develop.

PAAG consists of 97.5% loosely bound water molecules, which are easily exchanged with the surroundings, i.e., the extracellular matrix. Two different type observations support this. In one, methylene blue was added to the gel, which after having been injected subcutaneously in the abdomen of minipigs, disappeared within 1 day (own unpublished observations). The other showed that

radioactively labeled water molecules in PAAG were quickly exchanged with surrounding water (J. Brahm, personal communication, 2007). Such a dynamic exchange of water molecules would provide the gel with a constant undulating motion on a microlevel, preventing not just capsule formation but also diffuse fibrosis—a problem that has been described for some of the more static bulking agents such as the polypropylene mesh used in urine incontinence[28] and the combination gels (suspensions consisting of solid particles and a transient carrier gel).[19,24]

The observation that PAAG, unlike silicone gel, is integrated into the host tissue by ingrowth of a vessel-bearing fibrous network is new, but vessel ingrowth into the PAAG has been described before in a rabbit study.[29] Furthermore, studies on human facial soft tissue augmented with 1- to 2-cm^3 deposits of PAAG have shown that these have obtained full integration with a mature network at 2 and 3 years.[19] In contrast, large deposits of 200 to 300 cm^3, e.g., for breast augmentation or body sculpturing purposes, still retain some free gel after 8 1/2 years,[10] and serious complications, which needed surgical and antibiotic treatment, have been described several years after the injection.[14–16] An explanation of this phenomenon may be that PAAG deposits of this size, if not contaminated during the injection, may have been contaminated at a later time, giving rise to a low-grade infection. The network buildup starts peripherally, and the smaller the deposit the faster and more effective the tissue integration with ensuing antibacterial effect from circulating blood leukocytes.

In contrast to silicone gel, which was seen within most histiocytic cells of the gel deposits, PAAG of the types used in this study was not normally observed within these cells. However, we saw small collections of gel-laden macrophages beneath the capsule of regional lymph nodes during the early stages of gel/tissue integration, which must have originated from the injection site. These macrophages were only seen at 1 and 1 1/2 months and not at any later time, suggesting the early transient clearance of a minute inevitable fraction of less firmly bound polymer (R. Smith, Chempilots, personal communication, 2007). In accordance with this we saw enlarged lymph nodes with a thick subcapsular rim of macrophages without gel remaining at 3, 3 1/2, 4, and 6 months and normal-size lymph nodes and no subcapsular histiocytosis at 12 and 14 months.

A more prominent migration to regional lymph nodes has been described for silicone gel, especially in women with silicone breast implants.[30] This product was described histologically as partially empty "droplets" of a slightly refractile, peripherally located material within the tissue or lying within macrophages and foreign-body giant cells, just as we found in this study. It seems as if the silicone gel migration by macrophages occurs at a slower pace than the transient PAAG migration. We found just a few scattered sheets of macrophages distended by silicone (Figure 9) appearing no earlier than 12 months. However, others have described a more significant spread to regional lymph nodes years after tissue injection or silicone gel implant insertion, although further spread to the RES was considered negligible.[31]

Conclusion

By injecting PAAG and silicone gel into breast subcutaneous tissue, the urethral, the bladder, or the anal wall of 41 pigs, and euthanizing them at 1, 1 1/2, 3, 3 1/2, 6, 12, and 14 months, the gel–host interaction was examined, not just locally on muscle and connective tissue but also on regional lymph nodes and organs of the RES. In contrast to silicone gel, which was surrounded by a thick fibrous capsule, PAAG was anchored to host tissue by thin strands of a fibrous network. First, macrophages and giant cells entered the gel and then thin fibers of connective tissue emerged, and finally, a delicate network of mature connective tissue formed and the cells disappeared. A transient, early migration of a minute amount of gel, probably representing a less firmly bound fraction of the polymer,

occurred for PAAG, whereas incipient silicone gel migration was seen at a later time.

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COMMENTARY

As we are exposed to more of the permanent fillers from Europe, it becomes increasingly important to understand the histology of tissue integration and its ramifications of potential infection and foreign-body granulomatous reactions. This elegant basic pathology study is a help in our understanding of the pros and cons of long-lasting fillers.

GARY MONHEIT, MD
Birmingham, AL

CHRISTENSEN et al.

The effects of polyacrylamide hydrogel in normal and osteoarthritic animal joints.

Osteoarthritis Research Society International (OARSI), World annual congress, Posters (2016, The Netherlands)

THE EFFECTS OF POLYACRYLAMIDE HYDROGEL IN NORMAL AND OSTEOARTHRITIC ANIMAL JOINTS

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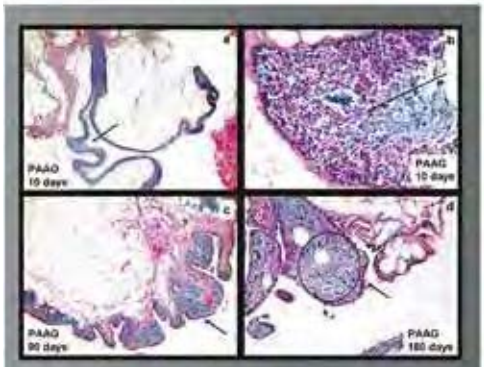
PURPOSE: Aim of this histopathological study was to investigate if intra-articular injection of polyacrylamide hydrogel (PAAG) is integrated into synovial tissue in normal and OA animal joints, and if this integration is sustained.

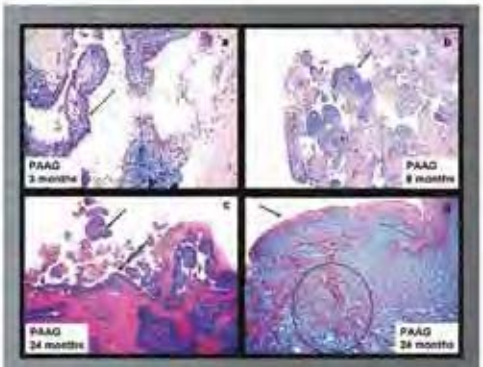
METHODS: A prospective, controlled, longitudinal study of normal knee joints injected with PAAG was performed in 10 rabbits up to 1 year.
Post mortem examination was carried out in 18 OA horse joints, which had previously been treated with PAAG for up to 2 years.



Rabbit synovialis after10 (a,b) and 30 days (c,d). At day10 the gel was still in the cavity (a,b). The integrated gel is blue with a fibrous net. (circle)

RESULTS: Integration of the injected gel was evident at day 10 in the rabbit and by day 14 in the horse with proliferation/invasion of synovial cells into the gel.

By day 90 in rabbit joints and day 30 in horse joints, the gel had formed a thick membrane with a thin fibrous network and covered by a 2-3 cell thick synovial lining facing the joint cavity. This pattern persisted for up to at least 2 years.



Horse joint after 3 and 8 months (a, b) and after 2 years (c, d). The surface is marked with arrows, the fibrous network with a circle.

CONCLUSION: **Intra-articular injection of PAAG results in a stable, long-lasting subsynovial layer of gel traversed with thin strands of connective tissue. Further studies to explore the potential effect of this layer on OA are warranted.**

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CHAPTER 3

CRITICAL OA CASE

JANSSEN, KOENE, LISCHER

Intraarticular application of polyacrylamide hydrogel as a treatment of osteoarthritis in the distal interphalangeal joint: case series with 12 horses.

Pferdeheilkunde (2012) 28:6 650-656

BATHE et al.

Intra-articular polyacrylamide hydrogel for the treatment of 20 horses with non-responsive osteoarthritis of the interphalangeal joints: a prospective study.

Veterinary Orthopedic Society 43rd Annual Conference Abstracts (2016, USA)

JANSSEN, KOENE, LISCHER

Intraarticular application of polyacrylamide hydrogel as a treatment ***of osteoarthritis in the distal interphalangeal joint: case series with 12 horses.***

Pferdeheilkunde (2012) 28:6 650-656

Pferdeheilkunde 28 (2012) 6 (November/December) 650-656

Intra-articular use of Polyacrylamide hydrogel as a treatment for osteoarthritis in the distal interphalangeal joint: a case series of 12 horses

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Abstract

Treatment of osteoarthritis in the distal interphalangeal joint is a challenge when conventional methods of intra-articular treatment prove unsuccessful. The intra-articular use of polyacrylamide hydrogel offers a new treatment approach. This substance has been successfully used in human plastic and aesthetic surgery as a soft-tissue filler with a long-lasting effect. In this case series, 1 mL of polyacrylamide hydrogel was injected into each of the affected distal interphalangeal joints of 12 horses. All of the horses had been suffering from chronic recurrent lameness — caused by osteoarthritis — in one or both of the forelimbs for at least three months. The horses had been previously and unsuccessfully treated at least twice with one or more conventional therapies (hyaluronic acid, glucocorticoids, autologous conditioned serum). The diagnosis of osteoarthritis was established on the basis of clinical findings, block anaesthesia and intra-articular anaesthesia, as well as radiographic and MRI examinations. Clinical follow-up examinations were carried out after one and six months. No adverse reactions were observed in any of the 12 horses treated. Six months after treatment, 8 horses were no longer lame, 2 horses showed an improvement in their condition, and no change was observed in 2 horses.

Keywords: Horse / osteoarthritis / lameness / MRI / distal interphalangeal joint / orthopaedics

Introduction

Osteoarthritis (OA) or arthritis (synonym) of the distal interphalangeal joint is one of the most common causes of chronic-intermittent and progressive lameness in the forelimbs of sport horses aged between 4 and 15 years (Lowe 1976, Pool et al. 1989, Wright 1993). OA of the distal interphalangeal joint is characterised by an increase in joint distension, synovitis, joint pain, subchondral bone remodelling and degeneration of the joint cartilage (Goldring and Goldring 2007, Hertsch and Maaß 2009, Hunziker 2001). The causes include acute or repetitive mechanical and traumatic factors, such as distortion, collateral ligament damage, unequal loading of the joint due to malpositioned hooves and/or limbs (Baxter et al. 2011, Raker et al. 1966) and joint infections. OA of the distal interphalangeal joint may also occur as a consequence of or in connection with aseptic podotrochlosis (Hertsch and Maaß 2009).

Joint inflammation leads to synovitis and capsulitis (Howard and McIlwraith 1993) and, subsequently, to persistently elevated intra-articular pressure. It is also postulated that impaired venous blood flow and/or an enrichment in proteins with oncotic properties in the cancellous bone increases the intra-osseous pressure in the subchondral bone (Arnoldi et al. 1972, Arnoldi et al. 1979, Arnoldi et al. 1980, Bünger et al. 1983, Gronlund et al. 1984). These phenomena ultimately lead to irreversible, chronic degenerative changes in the joint cartilage (Blake et al. 1989, Levick 1990). These pathophysiological changes result in a vicious circle, maintaining the joint inflammation.

Depending on its severity, lameness caused by OA of the distal interphalangeal joint may be stopped by low palmar nerve block. Intra-articular distal interphalangeal joint anaesthesia is useful for diagnosis if examination occurs within a few minutes of injecting approximately 5 mL of local anaesthetic. If left longer, of if higher doses of local anaesthetic are used, diffusion will render the results too imprecise.

Dyson 1998, Rijkenhuizen 2001, Schumacher et al. 2000). Measurement of increased joint pressure has been described as a diagnostic aid (Zuther and Hertsch 2004). However, this method has not yet been adequately validated.

The radiological signs of OA in the distal interphalangeal joint are sclerosis of the subchondral bone, osteophyte and enthesophyte formation, and a narrowing or complete obliteration of the joint cavity (Baxter et al. 2011). In the absence of any radiological evidence, cartilage changes, subchondral bone pathologies, increased joint distension and a thickened joint capsule can be revealed by magnetic resonance imaging (Baxter et al. 2011, Bell et al. 2009, Sill 2007). Conventionally, the distal interphalangeal joint is treated with hyaluronic acid, glucocorticoids and polysulphated glycosaminoglycan (PSGAG) (Ferris et al. 2011, Frisbie et al. 2009, Goldberg and Buckwalter 2005, Higgins and Lees 1984, Michon et al. 2010, McIlwraith 2010). A newer therapeutic approach is the use of Autologous Conditioned Serum (IRAP®) (Hrahaet al. 2011, Meijer et al. 2003). Surgical methods — neurectomy and arthrodesis — can be used as a last resort in the case of advanced and refractory OA of the distal interphalangeal joint (Fürst and Lischer 2012, Lischer and Auer 2012).

Intra-articular treatment with polyacrylamide hydrogel (PAAHG) offers an alternative treatment option. PAAHG (ArthramidVet®) is composed of 97.5% sterile water and 2.5% polyacrylamide polymer. This produces large and stable molecules with elastic, non-resorbable, non-toxic and viscous properties (Christensen et al. 2003, de Cassia Novaes and Berg 2003). PAAHG has been successfully used for over 10 years in human plastic and aesthetic surgery as a soft-tissue filler with a long-lasting effect (Breiting et al. 2004, Yagi et al. 2009). Stress incontinence in women has been treated by transurethral injection of PAAHG; treatment was successful in 68% of the patients (Lose et al. 2006). Because of its properties, the idea arose — during a discussion between a medical doctor and a veterinarian — of testing the use of PAAHG in horses with OA.

The objective of this pilot study was to investigate intra-articular PAAHG treatment in horses with chronic OA of the distal interphalangeal joint. The therapy was given exclusively to horses with a reliable diagnosis and in which therapy using conventional intra-articular medication had repeatedly proven unsuccessful.

Animals, Material and Methods

The horses treated were from the 2008 to 2011 caseload of the veterinary clinic for horses in Lüsche. Their average age was 10 years, with an age distribution of 4 to 14 years (Table 1). The 9 geldings, 2 stallions and 1 mare were exclusively used for showjumping and were all warmbloods except for one pony.

Clinical investigation

Prior to treatment with PAAHG (ArthramidVet®), lameness was assessed when walking and trotting, both in a straight line and in a circle, and on hard and soft ground. The assessment was based on the MEP lameness grading system of the American Association of Equine Practitioners, which comprises 5 grades of lameness (Dyson 2011). Distal interphalangeal joint distension was graded from 0-4 (0 = absence; 1 = mild; 2 = moderate, 3 = severe; 4 = massive joint distension). In one horse (case 10) with bilateral lameness, only the most severely lame limb was taken into consideration. Lameness improved by at least 50% in all of the horses following low palmar nerve block. The distal interphalangeal joint block performed the following day using 5 mL of Mepivacain (Scandicain®) improved lameness by at least 50% after one minute. The clinical investigation, treatment and follow-up were carried out by an experienced orthopaedic surgeon. In the initial examination, two X-ray images were taken of the distal interphalangeal joint (lateral and dorsopalmar view of the pastern and coffin bones (Oxspring view)), and an MRI scan of the toes of both forelimbs was performed.

Table 1 *Details of cases in the study*

Case No.	Age	Gender	Breed	Limb	Previous treatment of the distal interphalangeal joint
1	10	Gelding	WB	RFL	Glucocorticoids; Hyaluronic acid
2	4	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid
3	13	Gelding	Pony	LFL	Glucocorticoids; Hyaluronic acid
4	6	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid
5	14	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid; ACS
6	8	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid
7	12	Stallion	WB	LFL	Glucocorticoids; Hyaluronic acid
8	11	Mare	WB	LFL	Glucocorticoids; Hyaluronic acid; ACS
9	10	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid
10	11	Stallion	WB	LFL+RFL	Glucocorticoids; Hyaluronic acid; ACS
11	6	Gelding	WB	LFL	Glucocorticoids; Hyaluronic acid
12	8	Gelding	WB	RFL	Glucocorticoids; Hyaluronic acid; ACS

WB = warmblood; RFL = right forelimb; LFL = left forelimb; ACS = Autologous Conditioned Serum (IRAP®).

Magnetic resonance imaging

The distal forelimbs were examined using a low-field MRI veterinarian limb scanner (0.27 Tesla), manufactured by Hallmarq (Guildford, England). The horses were sedated with 0.02 mg/kg Detomidine (Cepesedan®) and 0.02 mg/kg Butorphanol (Torbugesic®). The following investigation protocol was selected: T1 3D Sagittal; T2*3D Sagittal; T1 3D Frontal; T1 3D Transverse; STIR Gradient Echo Sagittal; STIR Gradient Echo Frontal; T2*3D Transverse; T2* Gradient Echo Transverse; T1 Gradient Echo Frontal High Resolution; T2 Fast Spin Echo Transverse; T2 Fast Spin Echo Frontal.

Inclusion criteria

All patients had been suffering from forelimb lameness for at least three months and had OA in the distal interphalangeal joint in one or both forelimbs, with significantly increased joint distension. The joints had been unsuccessfully treated at least twice with intra-articular injections of hyaluronic acid, glucocorticoids and/or Autologous Conditioned Serum (IRAP®). The initial treatment involved the fitting of an orthopaedic shoe, intended to shorten the toe, ensure a good rolled toe and correct any medial lateral imbalance. All patients were subjected to an MRI scan of both forelimbs. At least two of the following criteria had to be met: significantly increased joint distension, subchondral bone changes, cartilage damage, bone cyst with joint involvement, increased fat-suppressed signal intensity in the subchondral bone of the distal pastern bone (Figure 1, a-c).

Exclusion criteria

Horses having developed OA as a result of a joint infection, or with skin diseases at or around the site of injection into the distal interphalangeal joint, were not included. Additional exclusion criteria were previous arthroscopy of the distal interphalangeal joint, and additional significant MRT findings, such as desmitis of the collateral ligament and/or tendinopathy of the deep digital flexor tendon.

Treatment

1 mL of ArthramidVet® was injected into each distal interphalangeal joint under sterile conditions. The skin was prepared by clipping or shaving, then washed with an iodine scrub (Degraseptin®) and disinfected with alcohol (Kodan®).

Instructions for post-treatment care

The horseshoe fitted during initial treatment was not changed. The horses were given 5 days of box rest, and were then walked for 20 minutes, twice daily for two weeks. The horses were then ridden at a walk and trot for 1 week. After the first follow-up examination, all of the horses were lightly exercised for a month, and the workload was then individually adapted to each horse. Joint distension and lameness were assessed one month and six months after the intra-articular injection of PAAHG.

Results

Before treatment with PAAHG, the mean lameness grade of the horses in a straight line was 1.8 (1-3), and the mean joint distension score was 1.75 (1-3) (Table 2). In 2 horses, the radiological examination produced no findings. Half of the horses showed slight radiographic signs of OA, 3 horses moderate signs, and 1 horse advanced signs. In one horse (case 8), the Oxspring view allowed the diagnosis of a pastern-bone cyst with distal interphalangeal joint involvement.

The MRI scan revealed increased distension of the distal interphalangeal joint in all of the horses. Subchondral bone changes were observed in 10 horses and cartilage defects in 11 horses. 3 horses had a pastern-bone cyst with distal interphalangeal joint involvement (Figure 1, a-c). The MRI scan revealed an elevated fat-suppressed signal intensity in the distal pastern bone (sagittal crest) in 4 horses (Table 2).

There were no problems injecting the hydrogel into the distal interphalangeal joint, and none of the horses suffered any undesirable effects.

Follow-up examinations

1. Follow-up examination (1 month)

One month after treatment, mean lameness had been reduced by one grade (initial: 1.8, min. 1, max. 3; 1 month: 0.8, min. 0, max. 2). Four horses (33%) were no longer lame, 3 had reduced lameness, and lameness remained unchanged in 4 horses. The condition of one horse had deteriorated. Mean joint distension was also reduced (initial: 1.75, min. 1, max. 3; 1 month: 1, min. 1, max. 1) (Table 2).

2. Follow-up examination (6 months)

After six months, the mean lameness grade was 0.3, min. 0, max. 1. Eight horses were no longer lame and were by this time once again being used for showjumping. In two horses, there was a significant improvement in lameness, and lameness remained unchanged in a further two horses, one of which (case 12) subsequently underwent neurectomy. Mean joint distension was 0.6, min. 0, max. 1.

Discussion

In this study, 8 of the 12 horses (67%) with OA in the distal interphalangeal joint, having been unsuccessfully treated with hyaluronic acid, glucocorticoids and/or ACS (IRAP®), were no longer lame six months after a single intra-articular injection of 1 mL ArthramidVet®. Treatment success following the administration of intra-articular medication in horses with OA in the distal interphalangeal joint varies from 50% to 89%, depending on the study (Gutierrez-Nibeyroet al. 2010, Jöstingmeier 2009, Songkhla 1997).

Jöstingmeier (2009) compared a combination of sodium hyaluronate (Hylartil®) and betamethasone (Celestovet®) with ACS (IRAP®). Inclusion criteria for this field study were lameness of the forelimb with a positive response to low palmar nerve

block and/or a positive response to distal interphalangeal joint anaesthesia, as well as radiographic changes displaying OA in the distal interphalangeal joint. Six months after the initial treatment, 54 horses (76%) were no longer lame. In the sodium hyaluronate/betamethasone group (n=27), 63% of the horses were no longer lame after an average of 2.8 joint injections, whereas in the ACS group (n=27) 89% of the horses we no longer lame after an average of 3.3 injections.

In a retrospective study of 56 horses with foot pain, 20 horses were diagnosed with sinovitis/OA in the distal interphalangeal joint. Following intra-articular treatment with triamcinolone (6 mg/joint) and hyaluronic acid (20 mg/joint), 10 of these 20 horses (50%) recovered full use within a year, and displayed no lameness for at least three months (Gutierrez-Nibeyroet al. 2010). In an assessment of distal interphalangeal joint treatment with Hylartil® or combined Hylartil® and Celestovet® in 130 horses, the success rate 6 months after the last injection was 68.4%, with an average of 1.98 treatments per joint (Songkhla 1997).

In contrast to the abovementioned studies, OA in the distal interphalangeal joint in the 12 horses of this study was diagnosed by means of complex clinical, radiographic and MRI examinations. This also made it possible to exclude horses from the study that were shown to have other lesions in the deep digital flexor tendon or in the collateral ligaments. These defects could not be taken into account in similar studies involving only clinical and radiographic examinations.

The clinically established treatment success rate after six months is all the more significant in comparison with the abovementioned studies, considering that all of the horses had received unsuccessful intra-articular treatment at least twice, and the MRI scan showed degenerative lesions in all of the distal interphalangeal joints. Despite the prognosis in the case of cysts with joint involvement being uncertain, the 3 patients with a bone cyst in the pastern bone with distal interphalangeal joint involvement were also surprisingly no longer lame in the last follow-up examination. Two of these three horses had the highest lameness grade (3/5) and the most severe joint distension (3/4) of this study. Lameness had already disappeared in both horses within 1 month, and this remained the case up until the last clinical follow-up examination. This study raises the question as to whether PAAHG is capable of further improving the treatment success rate of existing synovitis, and, if so, for how long. However, it is also important to know what the exact indication for this form of therapy was, as two other horses in this study showed no improvement whatsoever.

The mode of action of PAAHG is unclear, since this drug has not yet been used in intra-articular therapy. An effect lasting over 2 years — as a non-resorbable soft-tissue filler in plastic surgery — has been described in a study on 101 patients (von Buelow and Pallua 2006). Long-term results concerning PAAHG breast implants show the substance to be stable, nondegradable and resistant to diffusion (Christensen et al. 2003). The viscoelasticity of PAAHG may have a shock-absorbing effect in the joint, with the large and stable molecule acting to «cushion» the impact when the joint is exerted. A positive effect was also observed in some horses with pronounced cartilage damage. It is possible that the hydrogel covers the subchondral mechanoreceptors; this intra-articular «nerve coating» would explain the reduction in lameness. The mode of action of PAAHG in the area around the bone cyst may consist in the closure of the cyst opening by the hydrogel. Further penetration of synovial fluid is therefore not

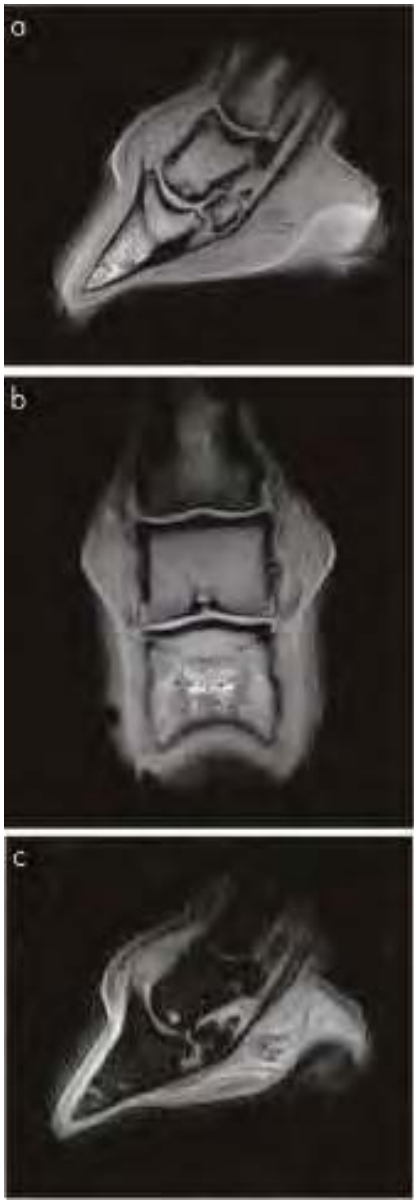

Figure 1. Sagittal (T1 3 D Sagittal) (A) and Frontal (T1 3 D Frontal) (B) MRI scan of the distal foot of the left front limb show a bone cyst in the distal aspect of the second phalanx with sclerosis, articular cartilage defect and involvement with the distal interphalangeal joint (left=lateral). Fat-suppressed image (STIR Gradient Echo Sagittal) (C) indicates increased signal intensity in the second phalanx and significant distension of the DIP joint.

possible, resulting in a less pronounced pressure increase and significantly less inflammation in the cyst. Chemical curettage can probably be excluded as a mode of action, since PAAHG is completely inert.

In this study, no undesirable effects resulted from the intra-articular injection of PAAHG. This observation was also confirmed by repeated MRI and radiographic follow-up examinations. Compared with the initial examination, no reaction, subchondral bone changes or changes in the joint capsule were found in either of the two horses investigated, neither three nor six months

after intra-articular administration of PAAHG. For the time being, we conclude that PAAHG would not appear to cause any — or at least only very slight — tissue reactions.

This observation can only be confirmed through histological examinations. The unchanged MRI findings would suggest that it remains a palliative therapy, but does not cure OA. This raises the question as to how the long the palliative effect lasts; in this study, it could be observed for 6 months.

The results of this study are encouraging. However, further controlled studies (double-blind studies) are necessary in order to investigate the treatment success in comparison to other drugs, the long-term effects and the mode of action.

Table 2 *Diagnoses of radiographic and MRI examination, Results of lameness examination and follow-up*

Imaging examination								Clinical examination					
X-rays				MRI				Joint distension (score 0-4)			Lameness examination (score 1-5)		
Case	OA	OCLL	Joint distension	Subchondral bone	Bone cartilage	OCLL	STIR	LE	1 mth.	6 mth.	LE	1 mth.	6 mth.
1	+	-	+	-	+	-	-	3	1	1	3	0	0
2	+	-	+	+	+	-	-	2	1	0	1	0	0
3	+	-	+	+	+	+	+	1	1	1	2	1	0
4	-	-	+	+	-	-	+	1	1	0	1	2	0
5	+	-	+	+	+	-	-	3	1	1	2	1	1
6	+	-	+	+	+	-	-	1	1	1	2	2	1
7	+	-	+	+	+	+	+	2	1	1	2	0	0
8	+	-	+	+	+	+	+	3	1	1	3	0	0
9	+	-	+	+	+	-	-	1	1	0	1	1	0
10	+	-	+	-	+	-	-	1	1	1	1	1	1
11	+	-	+	+	+	-	-	2	1	0	2	1	0
12	-	-	+	+	+	-	-	1	1	0	1	1	1

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Intra-articular polyacrylamide hydrogel for the treatment of 20 horses with non-responsive osteoarthritis of the interphalangeal joints: a prospective study.

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Intra-articular polyacrylamide hydrogel for the treatment of 20 horses with non-responsive osteoarthritis of the interphalangeal joints: a prospective study

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Abstract

Introduction
Polyacrylamide hydrogel (PAAG) is a novel treatment for osteoarthritis (OA), but many previous case series have poorly defined clinical inclusion criteria. The aim of this study was to determine the efficacy of PAAG in equine arthritic interphalangeal joints, which had not responded to previous intra-articular treatment with corticosteroids.

Materials and Methods
Lameness was localized to the proximal/distal interphalangeal
joint by diagnostic analgesia; radiography/standing MRI was consistent with OA. After treatment with 1ml of PAAG (Arthramid Vet, Contura International) intra-articularly horses had 4 weeks of exercise restriction before a progressive return to ridden exercise. Follow-up was by re-examination and telephone survey.

Results
20 horses met the inclusion criteria. All were adult sport horses with persistent lameness after previous treatment with corticosteroids. The average lameness duration was 15mo and average lameness score was 3/10. 10 had arthritic changes evident radiologically. 18 underwent MRI examination and all had osteoarthritic changes. One horse was treated twice, and had a transient adverse reaction. Long term follow-up was available on 18 cases; with median follow-up of 12mo. 12/18 returned to full function; 3/18 to lower level and 3/18 failed to improve.
Discussion/Conclusion: The success rate and long-term duration is encouraging given the case severity. A control group would have been preferable, but each case could act as its own control, as conventional treatments had already failed. The only significant difference in the management of each case was the PAAG treatment. The method of action of PAAG is uncertain, and further work is required to study this.

Acknowledgement
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CHAPTER 4

PAAG & OTHER OPTIONS

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Efficacy and adverse effects of joint medication in the horse - A review of the literature - Part 2: Regenerative and innovative joint medication in the horse

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Abstract

Regenerative and innovative joint medication in the horse - Part 2: Efficacy and adverse effects of joint medication in the horse - A review of the literature

The pathogenesis of osteoarthritis in the horse is a complex and incompletely understood process of joint degeneration. Many cases have proven to be refractory to intra-articular medication using conventional therapeutic agents. Over recent years this has encouraged the development of several new techniques using regenerative preparations. The potential advantage of the use of these therapies is that they should allow a more complete restoration of joint health. In the future it appears likely that gene therapy will permit the regrowth and repair of damaged cartilage along with healthy joint fluid. The objective of this research is to identify the clinically relevant information about regenerative and innovative intra-articular joint medications such as Pentosan polysulphate, Autologous Conditioned Serum, Platelet-Rich Plasma, Stem Cell techniques and Gene Therapy based on a review of current in vitro and in vivo studies.

Keywords: osteoarthritis, joint injection, evidence, autologous conditioned serum, platelet-rich plasma, stem cells, gene therapy

Introduction

Intra-articular treatment of OA in both the horse and in humans has been the subject of medical research for many years. The pathogenesis of OA is still not fully understood, and there are currently no successful therapies on the market (Carmona and Prades 2009, Bay-Jensen et al. 2010, Michon et al. 2010). In addition to
the conventional joint medication discussed in Part 1 of this literature review, this second part gives an evidence-based presentation of further intra-articular therapeutic methods, based on the fundamentals presented in the introduction to Part 1.

Autologous Conditioned Serum

After TNF-α, IL-1 is the inflammatory mediator that plays the greatest role in the onset and perpetuation of a joint inflammation and the destruction of tissue. When IL-1 binds to the IL-1 receptor, MMPs, aggrecanases and PGE2 are activated (*McIlwraith* 2010). The IL-1 receptor antagonist protein (IL-1Ra) also binds to the IL-1 receptors, thus reducing the influence of IL-1 in OA in the horse.

The incubation of autologous blood with borosilicate glass beads, whose surface has been treated with chromium sulphate, stimulates the monocytes to increase IL-1Ra production (*Meijer* et al. 2003, *Hraha* et al. 2011). The autologous conditioned serum (ACS), enriched with these factors, can be removed by centrifugation after 24 hours and injected into the synovial structures. The kit for manufacturing the serum is marketed in Germany under the name «irap®» (IRAP I) or «ABPS®» (IRAP II) and has also been authorised for use in human medicine, marketed as «Orthokin®».

The growth factor and IL-1Ra content of the serum has been determined in vitro for the various kits and with different incubation times. The IL-1Ra content after 24 hours of incubation could be increased 93-fold using IRAP I, 119-fold using IRAP II and 82-fold in the control, for which a simple, untreated glass Vacutainer tube was used. In addition to IL-1Ra, the serum was also enriched with cytokines such as TGF-β (transforming growth factor beta) and IGF-1 (insulin-like growth factor 1) was also enriched. However, the content of the aforementioned factors in the serum was subject to strong individual variance (*Hraha* et al. 2011).

In horses with chip fractures of the scaphoid bone (EOFEM), a clinical improvement in symptoms was observed following ACS-therapy (4x6 mL in weekly intervals). Significantly less synovial hyperplasia and haemorrhaging were observed compared with the placebo group, as well as less cartilage fibrillation (*Frisbie* et al. 2005/2007). 35 days after the last ACS treatment, an elevated concentration of IL-1Ra could be found in the synovial fluid. However, this result was obtained using mouse antibodies and not a horse-specific ELISA kit.

According to one clinical report, 262 horses with OA, having already been pre-treated with corticosteroids or HA, were given ACS-therapy. They were given 2-3 injections at 8-12 day intervals (2 mL ACS) into various joints. After 6 weeks, 199 (76%) of the horses were no longer lame, and in 22 (8%) of the horses initial lameness was reduced (according to the AAEP score). 178 (68%) of the horses were also free of any limp at the 12-week follow-up (*Weinberger* 2008).

In a recent human medical study involving 376 probands with OA in the knee joint, and in which 134 of the subjects

were treated with ACS, 135 with HA and 107 with NaCl, the patients injected with ACS experienced a significant improvement in clinical symptoms and an improved quality of life (*Baltzer* et al. 2009). Therapy with autologous blood should be well-tolerated, and the studies described above are promising. No comparative academic studies are available on the success of synovial transplantation. However, in some cases, it is not possible to verify the growth factor and IL-1Ra content, which may explain the varying therapeutic success (*Hraha* et al. 2011).

Platelet-Rich Plasma

Platelet-Rich Plasma (PRP) that has been enriched with thrombocytes, growth factors and cytokines and is obtained from whole blood via centrifugation (plasmapheresis). The resulting PRP contains 2 to 8 times more platelets/µL than native blood plasma (1.1x106 platelets/µL) and is used for the treatment of wounds, tendinopathies, bone defects and OA (*Gonshor* 2002, *Miller* et al. 2007, *Textor* 2011, *Anitua* et al. 2012). A large number of growth factors, such as insulin-like growth factors 1 and 2 (IGF-1/2), epidermal growth factor (EGF) and connective tissue growth factor (CTGF), are secreted by the α-granules of the platelets (*Marx* 2004, *Blair* and *Flaumenhaft* 2009). By adding thrombin or calcium chloride, the platelets in the PRP can be activated. Through the release of coagulation factors, they then form an autologous platelet gel (APG) (*Del Bue* et al. 2008, *Fortier* et al. 2011).

In vitro studies conducted using pig and rabbit chondrocytes and human stem cells have shown that PRP promotes cell proliferation as well as the synthesis of proteoglycan and type II collagen (*Akeda* et al. 2006, *Mishra* et al. 2009, *Lee* et al. 2012). However, there have not yet been any scientific studies on the use of PRP therapy to treat OA in horses.

Up to now, *in vivo* studies and case reports on the horse mainly describe the successful use of PRP to treat tendon lesions or to promote the healing of wounds (*Carter* et al. 2003, *Maia* et al. 2009, *Bosch* et al. 2011, *Iacopetti* et al. 2012). An in-vivo model involving 15 sheep with defective cartilage in the knee joint concluded that a therapy combining microfracture surgery and PRP gel improved the outcome in the histological evaluation of the cartilage after 6 months, compared with microfracture surgery alone or combination with liquid PRP (*Milano* et al. 2010).

In human medicine, PRP was initially most widely used in oral surgery. However, there are now more and more reports concerning PRP therapy for OA in humans, though there are still few evidence-based studies on the subject (*Kon* et al. 2011, *Nguyen* et al. 2011, *Napolitano* et al. 2012, *Sánchez* et al. 2012, *Spaková* et al. 2012).

Besides the actual platelet content, the leukocyte content is also relevant in the practical use of PRP. Since leukocytes have the effect of catabolic enzymes, and their content can likewise be enriched during the manufacture of PRP, care should be taken when choosing products or a manufacturer of PRP, to opt for the highest possible platelet content and the lowest possible leukocyte content (*McCarrel* and *Fortier* 2009, *Sundman* et al. 2011, *Fortier* et al. 2011).

Mesenchymal stem cells

In theory, multipotent mesenchymal stem cells (MSCs) are able to further develop into different cell types of a specific lineage. The aim of MSC therapy is not only to repair, but also to regenerate, i.e. scar-free healing of destroyed tissue (*Koerner* et al. 2006, *Lee* and *Hui* 2006, *Frisbie* and *Smith* 2010). MSCs for intra-articular therapy are generally obtained from bone marrow from the sternum or hip bone, or from subcutaneous fat tissue (*Muschler* et al. 2004). In both procedures, the MSCs obtained are processed in the laboratory prior to injection into the joint. Depending on the method used, this takes between 2 days and 3 weeks. This cleans the substrate and allows the targeted proliferation of the MSCs, a process known as «culture expansion» (VetCell technique) (*Pittenger* et al. 1999, *Gutierrez-Nibeyro* 2011).

The production of MSCs was studied *in vitro* for both procedures, both in terms of the number of MSCs and progenitor cells produced, as well as their tendency towards chondrogenesis upon the addition of growth factors. With the enrichment of bone marrow derived MSCs, a total cell count in the millions can be achieved, whereas that obtained using adipose tissue is only in the order of a few hundred thousand (*Smith* et al. 2003, *Fortier* 2005, *Frisbie* and *Smith* 2010, *McIlwraith* 2010). The observed cell-differentiation and chondrogenesis potentials of the cultivated adipose-derived MSCs were also lower than those of the bone marrow MSCs (*Kisiday* et al. 2008, *Vidal* et al. 2008).

Current *in vitro* research focuses mainly on optimising isolation of the MSCs. While «culture expansion» is currently achieved via the adhesion and proliferation of the cells on the surface of tissue cultures, in the future it should be possible to isolate the MSCs more selectively with the aid of cell-surface antigens. In this way, fewer other cells, such as fibroblasts, will be cultivated and subsequently injected into the joint (*Taylor* et al. 2007 *Frisbie* and *Smith*, 2010).

Based on the findings of the *in vivo* model (EOFEM) (*Frisbie* et al. 2009b), the authors do not yet recommend intra-articular MSC therapy. This model compared adipose-derived and bone marrow-derived stem cell therapy, concluding that the bone marrow-derived MSC therapy gave better results. However, even these were not significant enough for a treatment recommendation to be considered.

In vivo meniscectomies, carried out on 12 goats to induce OA, demonstrated the good regeneration potential of the menisci following intra-articular injection of MSCs (6 weeks after the meniscus was damaged), resulting in less pronounced secondary OA (*Murphy* et al. 2003). The positive effect of bone marrow-derived MSCs on the soft tissue of the menisci is also described in a case study in human medicine (Centeno et al. 2008). Based on these findings, the success of MSC therapy was studied in a prospective, multicenter trial, which focused particularly on meniscus damage in the horse (*Ferris* et al. 2009). After a follow-up examination at 21 months, 77% of the 39 horses treated with bone marrow-derived MSCs could be used again. 36% of the horses recovered or even surpassed their former level of performance (*Ferris* et al. 2009). Of the 39 horses, the knee joint was affected in 29 cases, and 20 horses had arthroscopically diagnosed meniscus damage. Following

arthroscopy and stem-cell therapy, 60% of these horses were able to recover a certain level of performance, whereas the success rate following exclusively surgical therapy was only 47% (*Walmsley* et al. 2003).

There is evidence that MSCs show tropism towards damaged cells (such as fibrillated cartilage or menisci) and that therapy after 14 days (see EOFEM) is probably too early (*Luyten* 2004, *Fox* et al. 2007, *Frisbie* and *Smith* 2010). Controlled in-vivo studies have however so far failed to demonstrate a significantly beneficial effect of MSCs on OA in the horse. On the basis of present knowledge, MSC therapy for OA in the horse may therefore not yet be recommended.

The use of MSCs in the surgical treatment of cartilage defects, directly following subchondral bone microfracture, gave much more promising long-term results (*Fortier* et al. 2011, *McIlwraith* et al. 2010 *Frisbie*, 2011). In an in-vivo model, 12 horses with cartilage defects in the knee joint were treated with either microfracture surgery alone, or with concomitant MSC therapy. Significantly better regeneration of the cartilage matrix was seen in the group of horses treated with MSCs, according to both the arthroscopy repeated 3 months after treatment, and the macroscopic and histological evaluations and magnetic resonance imaging after 8 months (*Fortier* et al. 2011, *McIlwraith* et al. 2010 *Frisbie*, 2011). The therapeutic success of arthroscopic microfracture surgery combined with the intra-articular use of MSCs is therefore greater than for the injection alone.

Further therapeutic approaches

Gene therapy

Various institutions, such as the «Orthopaedic Research Society» and the «Orthopaedic Research Center» at CSU are committed to finding new strategies for the treatment of OA. One of the areas of current research is gene therapy.

For example, gene transfer increases the synthesis of IGF-1 and IL-Ra in equine synovioblasts. These cytokines have a beneficial effect on the treatment of OA, since they are able to inhibit inflammatory mediators such as IL-1 and TNF-α. (*Frisbie* 2005, *Frisbie* and *McIlwraith* 2005, *Haupt* et al. 2005, *Nixon* et al. 2005, *Goodrich* et al. 2006a, *Morisset* et al. 2007).

In vivo, production of IL-Ra was increased via the synovioblasts, using a harmless adenovirus to introduce the genetic sequence of IL-Ra into the joint. This gene therapy, administered to 8 horses with chip fractures of the MCJ, led to a significant reduction in the initial lameness. Compared with other therapies that are also being investigated using the EOFEM model (corticosteroids, PSGAG, pentosan polysulphate, HA, shockwave therapy), the high IL1-Ra content allows the most effective reduction — in both macroscopic and histological terms — of both clinical lameness and the progression of the induced OA (Frisbie et al. 2002, Frisbie and McIlwraith 2005).

Furthermore, a comparable model involving 12 horses (24 joints) showed that persistently elevated IGF-1 levels could be achieved intrasynovially by means of a viral vector (*Goodrich* et al. 2006a). This growth factor has an anabolic effect on cartilage metabolism, since it leads to gene activation in chondrocytes via specific receptors on the cell surface. A significantly higher level of IGF-1 mRNA was still found in the repair tissue 9 weeks after the transplantation of IGF-1-enriched chondrocytes into existing cartilage defects of the femoropatellar joint in 16 horses, compared with the transplantation of native chondrocytes. Furthermore, both in macroscopic and histological terms,
union of the defect occurred more rapidly and the type II collagen content was higher (Goodrich et al. 2007). The therapeutic success of combined anti-catabolic (IL-1Ra) and anabolic (IGF-1) gene modulation has already been confirmed in an *in vivo* model (EOFEM) (*Morisset* et al. 2007).

However, no market-ready products will be available in the immediate future, as a vector is yet to be developed that does not trigger an intrasynovial immune response and that prompts faster gene expression (*Goodrich* et al. 2006b, *Goodrich* et al. 2009).

Pentosan polysulphate

The pentosan polysulphate (PPS) in PSGAG is a plant-based heparinoid, obtained from the hemicellulose of the European beech (*Frisbie* 2006). The sodium salt of PPS serves as an antithrombotic and antilipemic agent. A calcium derivative is also used for the treatment of musculoskeletal disorders (*Little* and *Ghost* 1996).

In vitro tests have shown the synthesis of hyaluronic acid in human synovioblasts to be stimulated by PPS (*Costeseque* et al. 1986). An anabolic effect on damaged human and equine chondrocytes and increased synthesis of proteoglycan following the administration of PPS were also demonstrated (*Hutadilok* et al. 1988, *Frean* et al. 2002). Since the immune response, fibrin and lipids impair microcirculation in the subchondral bone in OA, therefore increasing the risk of avascular necrosis, the antithrombotic and antilipemic effect of PPS in the treatment of OA is also beneficial (*Ghosh* et al. 1992, *Cheras* et al. 1993, *Little* and *Ghost* 1996). Oral, subcutaneous, intramuscular, intravenous and intra-articular administration of PPS in the horse have been described (*Little* and *Ghost* 1996).

An in vivo model involving sheep showed improved joint function and reduced histological cartilage damage in the knee joint after weekly intra-articular administration of PPS over a 4-week period (*Ghosh* et al. 1993). As yet, in the horse, only the intramuscular administration of PPS (3mg/kg IM, weekly for 4 weeks) has been investigated in vivo, using the EOFEM model (*McIlwraith* et al. 2012). Compared to the placebo group, the treated joints showed significantly less cartilage fibrillation. However, an approved product is currently only marketed in Australia.

Polyacrylamide hydrogel

Polyacrylamide hydrogel (PAAG) consists of water (97.5%) and the polyacrylamide polymer (2.5%). It has been used in humans for several years to reinforce the subcutaneous tissue and to treat stress incontinence in women (*Lose* et al. 2006, *Pallua* and *Wolters* 2010). Human medical studies indicate good histocompatibility and a long-lasting effect, since the substance is assimilated into the soft tissue (*Fernández-Cossio* and *Castano-*

Oreja 2006, *Christensen* et al. 2003, *Christensen* et al. 2008, *Bello* et al. 2007).

Successful treatment of refractory OA in horses has been reported in initial clinical experiences. The first signs of improvement in the clinical symptoms (degree of lameness) could already be seen 4 weeks after a single injection of Arthramid ® Vet (2 mL) in 18 of the 19 horses treated (*Ankorina-Stark* and *Koene* 2011).

Conclusions

In equine medicine, unlike in human medicine, only very few controlled, randomised clinical studies exist that may be used to support the choice of treatment. Experimental *in vivo* models, such as the EOFEM of the midcarpal joint, therefore often provide the only alternative. Apart from the quality of the model compared with naturally-occurring OA, the number of probands and the financing of the studies by the manufacturer are further important details that must be taken into consideration when reviewing this type of academic work (*Richardson* and *Loinaz* 2007). In the absence of evidence, it is necessary to fall back on *in vitro* studies, which evaluate the effects of medicinal products using cell cultures or explants.

The choice of the appropriate treatment for a disease is more often based on the veterinarian's own experience than upon scientifically sound information (*Ferris* et al. 2011). This literature review summarises the latest scientific findings that have been published on the effectiveness of joint medication in order to provide the practising veterinarian with a substantiated scientific basis — from the available external evidence — for choosing a medical treatment for OA in the horse.

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CHAPTER 5

ALL PAAG ARE NOT THE SAME

NARINS, SCHMIDT
Polyacrylamide hydrogel differences: getting rid of the confusion.
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Polyacrylamide Hydrogel Differences: Getting Rid of the Confusion

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ABSTRACT

Polymer hydrogels have been used for many years in European and Asian countries, and these products are often considered to be the same material in different packaging. This, however, is not the case. Performance and safety profiles depend on many factors including chemical and physical characteristics (including rheological properties), manufacturing process and control (cross linking, impurities, stability, etc.), injection technique, and interaction with surrounding tissues. Polyacrylamide hydrogel (PAAH) products, although often considered equal, have clear differences in composition, manufacturing, and injection technique as well as ability to interact with surrounding tissues, characteristics that determine the safety and effectiveness profiles of each of these gels.

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INTRODUCTION

Polyacrylamide hydrogels (PAAH) have been used for many years in European and Asian countries, and they have been and some still are produced by a number of different manufacturers (Table 1). None of the products are currently marketed in the U.S., but a pivotal clinical study of Aquamid (Contura International A/S, Denmark) has recently been completed in the U.S.

The PAAH products are often considered to be the same material in different packaging, but this is not the case. There are important differences in the composition of the hydrogels, their manufacturing and control, injection technique used to implant the gels, and their ability to interact with the tissue into which they are injected. All these factors are likely to impact the performance and/or safety profile of the PAAH product.

Different polymer hydrogels have different chemical and physical structures: Bio-Alcamid (Polymekon, Italy), for example, is reported to be composed of polymers of alkylimide units;[1] Evolution (ProCytech, France) is composed of positively charged polyvinyl microspheres suspended in an acrylamide polymer;[2] Aquamid is composed solely of a cross-linked polyacrylamide.[3] Moreover, there are different cross-linking techniques applied for the various hydrogels, which have a significant impact on the physical and chemical product characteristics and thereby on the tissue interaction and longevity. Information on details regarding stability, visco-elastic properties, pH, and dry matter content of different PAAH products is sparse and stems mainly from the manufacturers. Among the PAAH products, Interfall, appeared at the market in the early 90s and was invented and patented in Ukraine. The first world conference on its application was held in Kiev in November 2000. Subsequently a number of similar preparations emerged: Formacryl, Bioformacryl, Cosmogel, Argiform (from Russia), and Amazingel (from China).

In the following, three of the best known and most widely used hydrogels will be more thoroughly described with respect to these characteristics, and their differences will be highlighted in Tables 1 and 2. Unless otherwise indicated, all analyzes have been carried-out by Chempilots A/S, Farum, Denmark.

Characteristics of Different Hydrogels

Bio-Alcamid

History

The gel started out as Formacryl, a product manufactured by the Russian company, Bioform, but in 2000, the product was manufactured by the Italian company, B&B Dental SRL (Progen, now Polymekon) and the name changed to BioFormacryl—the forerunner of Bio-Alcamid.

Bio-Alcamid was launched in late 2001. The reported composition of Bio-Alcamid varied but according to several publications and Polymekons homepage, Bio-Alcamid contains synthetic cross-linked amide-imide alkyl type polymer that does not contain any free monomers.[4-6]

Characteristics

Throughout 2002 to 2007, we have analyzed 17 samples of Bio-Alcamid for variables that we consider important for the function and safety of a hydrogel filler, including dry matter content, pH, rheological properties, and chemical composition. The dry matter content varied from 3.4 to 4.9 wt-%, and the pH was distinct acidic in the range of 3.3 to 5.7. A rheological analysis revealed major variations in the elastic modulus: For Bio-Alcamid lips, the modulus varied within 48–89 Pa (1 Pa= 10 dyn/cm^2), for Bio-Alcamid face modulus was 17–230 Pa, and for Bio-Alcamid body it was 113–229 Pa. Analysis of the chemical composition by infrared (IR) spectroscopy, ^{1}H- and ^{13}C-NMR, and Raman spectroscopy was performed twice on selected samples. The presence of imide groups could not be identified whereas we identified polyacrylamide in all Bio-Alcamid samples.

In a further analysis by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES) (Risoe National Laboratory, Roskilde, Denmark), we found that the sulphur content in Bio-Alcamid was as high as 709 ppm as compared with 4 ppm in another PAAH, Aquamid. Finally, two samples of Bio-Alcamid, one sample of Aquamid, and a 4 wt-% commercial available polyacrylamide powder from Sigma-Aldrich in sterile water (CP batch no. 144355) were analyzed by IR spectroscopy and ion chromatography. Residual sulphate and ammonium was detected in the Bio-Alcamid products but not in the Aquamid gel and not in the 4 wt-% solution of polyacrylamide. The pH of the two Bio-Alcamid products was low, below 3.5 (lips) and below 4.5 (face), respectively. The corresponding pH of Aquamid was 7.5, identical to that found in the polyacrylamide solution.

Gel-Tissue Interaction

Bio-Alcamid is indicated for corrections of soft tissue defects and contour deformities of the face and body. This indication includes treatment of HIV-associated lipoatrophy, redesign of jaw, chin and nose, repair of body muscles following trauma, postpoliomyelitis amyotrophy of calves, Parry-Romberg syndrome as well as rejuvenation of face and body (e.g., increased gluteal volume).[6-8] The product is supplied in 3 and 5 ml syringes and is injected subcutaneously using a 14–18 gauge needle and a single puncture hole. The needle is redrawn and reinserted in different tunnels to lodge each deposit until the desired volume is obtained. Each deposit may vary in size from 1–3 ml to 10–15 ml and the total volume injected can reach 250 ml, depending on the indication.[6,9,10]

Bio-Alcamid gel does not appear to integrate with adjacent tissues, but it induces the formation of a thin fibrous capsule, which surrounds the hydrogel.[5,11] The process commences within a few days of injection and is completed after 14 days in the mouse.[12] In humans, electron microscopy shows that a 0.5–1 ml deposit is completely encapsulated after three months.[11] Bio-Alcamid is thus considered an endoprosthesis due to the encapsulation process, which supposedly isolates the injected material from surrounding host tissues.[12]

Amazingel

History

Manufactured by FuHua high Molecular Matter Company, Ltd. and commercially available since 1998, Amazingel was registered in China in December 2000. The product was delivered as a 3–5 wt-% polyacrylamide hydrogel in 1 ml syringes and in 15 ml, 25 ml 50 ml, and 100 ml vials and was widely used for large-volume injections into breasts, buttocks and legs—in some cases with severe complications, mainly abscess formation.[13] Due to the risk of these complications, the product was banned in China in April 2006 but is still marketed in other Asian countries.

Characteristics

As for Bio-Alcamid, we had the opportunity to test Amazingel. From 2001 to 2006 we received and analyzed four samples of Amazingel. The product differed from Bio-Alcamid and Aquamid by being slightly opaque and a little fragmented. The elastic modulus was recorded in two samples to be 197 Pa (grey lid) and 418 Pa (white lid). The pH was 7.7 and 7.4, and dry matter content was determined to 3.1 and 3.5 wt-%, respectively. Two samples of the hydrogel (marked breast and wrinkles) were subjected to ^{1}H- and ^{13}C-NMR spectroscopy. The spectrum recorded on the breast sample demonstrated a polyacrylamide structure, whereas the NMR result on the wrinkle sample did not clearly identify the product as polyacrylamide.

Gel-Tissue Interaction

The gel has mostly been mentioned in connection with large volume treatments such as breast and buttock augmentation. One histology study has been carried out,[14] but it does not explain how the hydrogel interacts with surrounding tissue. However, with an elasticity modulus similar to or higher than Bio-Alcamid and an equivalent dry matter content to that of Bio-Alcamid, one would expect the same tissue interaction of the two products (i.e., the formation of a fibrous capsule around the hydrogel).[5,11,12]

Aquamid

History

Aquamid is manufactured and distributed by Contura International A/S, Denmark. The hydrogel was approved and CE-marked in Europe in 2001 for soft-tissue augmentation and is now available in several countries in Europe, Asia, the Middle East, Australia, and Latin America. To date, more than a quarter of a million people have been treated.[3]

Characteristics

The hydrogel is claimed to be a stable, non-degradable, cross-linked polyacrylamide gel with 2.5 wt-% dry matter (Table 2). Each batch is analyzed for consistency and compliance with product specification, including narrow tolerances of elasticity modulus, pH and impurities, and degradation products from the polymerization reaction (Table 2). Six lots of Aquamid 1-ml prefiled syringe were analyzed in 2001. The elasticity modulus was found to be in the range of 38–53 Pa, pH was 7.4–7.8, and the dry matter content in the range of 2.4–2.8 wt-%. IR and ^{1}H- and ^{13}C-NMR analyzes demonstrated the presence of polymeric acrylamide identical to a reference spectrum. Subsequently, a survey of Aquamid lots supplied during the period of 2000–2004 was summarized with the results that the elasticity modulus was in the range of 33–55 Pa, pH in the range of 7.3–8.2, and the dry matter in the range of 2.2–2.8 wt-%. From one lot an elementary analysis was conducted showing a sulfuric content of 4 ppm, sodium 3 ppm and phosphate 0.2 ppm.

Gel-Tissue Interaction

Cells have been found to grow well in the Aquamid gel,[15] and it allows vessel in-growth from adjacent tissues.[16] From an experimental study in porcine soft tissue (which is very similar to human soft tissues) and from 28 human soft tissue biopsies, it has been shown that the integration process will begin immediately after injection, and that a normal-size deposit of 0.2 ml has become fully integrated within 14 months.[16]

Manufacturing Process

Factors unique to the manufacturing process of each of the PAAH products confer specific properties to each product. For example, the visco-elastic properties of the final product are highly dependent on formulation and manufacturing processes. Products like Outline, Evolution, Beauticalm and Euthrofil are specifically manufactured to have a half-life of approximately 2 years,[2] whereas Aquamid, for example, is manufactured and marketed as non-degradable, permanent filler (Table 1).

While most products, including Aquamid, are marketed in prefilled single use syringes that minimize the risk of bacterial contamination, some hydrogels used for large-scale augmentation are also marketed in bottles and larger vials that are more prone to such contamination (Table 1)

Injection Technique

The visco-elastic properties of each hydrogel have a direct influence on the usability, i.e., the injection technique used to implant the product. "Softer" (low visco-elastic) hydrogels flow easier and can therefore be injected with thinner needles, whereas "harder" (high visco-elastic) hydrogels require larger needles and may also require different injection techniques (Table 2). A 'volume' technique where 1.5 to 5 ml or more of hydrogel is injected per site using a 14–18 gauge needle is typically recommended for products such as Bio-Alcamid, Amazingel, Argiform, and the 'Interfall', where the hydrogel needs to be placed as one lump of material. In the case of Bio-Alcamid, the injected hydrogel does not integrate with the surrounding tissue. Instead, it becomes covered with a very thin fibrous membrane (approximately 0.02 mm) that completely surrounds the hydrogel.[5,11] However, implantation of material in a large bolus has been associated with a higher incidence of complications, such as late-onset infections, granulomas, and hydrogel displacement.[13,17-28] Bio-Alcamid is no longer recommended for use in the Netherlands due to such complications.[29]

Aquamid is typically administered using a 27-gauge needle and a multi-line injection technique (retrograde fan-like injection). This series of small deposits facilitate tissue integration and vascularization of the implanted hydrogel, minimizing the risk of encapsulation, migration, and other late-onset complications such as biofilm-assisted infection.[16]

Safety and Performance

The safety and performance of Aquamid,[23-31] and to a lesser degree Bio-Alcamid[32,33] have been evaluated in clinical studies, for Bio-Alcamid exclusively on HIV-infected patients with facial lipo-atrophy due to anti-retroviral therapy. The literature shows little evidence of performance or safety of the other PAAH products,[34-36] but a widespread and largely indiscriminate use of these in some countries, predominantly eastern and middle-eastern countries, has caused serious long-term complications (mainly infection and migration) that have been described in several reports during the past ten years.[13,22-24,37-40] Because of these complications, Amazingel was banned from being used in China in 2006, where it is manufactured, but the product is still sold in several other Asian countries, and it is, like the Interfall gel, available over the internet.

Serious long-term complications are rarely reported in the follow-up of clinical trials, where products have been used under tight control and according to intended use.[22,29-31] However, as with any other product, when used incorrectly, some safety concerns may arise. For example, in a recent Japanese report on complications after injection with the PAAH Amazingel and Aquamid,[40] Aquamid had been used for treatment of contraindications in five of the eight patients who subsequently suffered complications attributed to the PAAG treatment. These contraindications include the following: injection into a site that had previously been injected with another permanent filler, injection into the lower eyelids, and injections as a bolus of material. Accordingly, one patient had previously been injected at the same site with another, non-degradable filler. Dermalive, two patients had the Aquamid hydrogel injected into their lower eyelids, and two had it injected into their cheeks as bolus injections instead of in a multi-line pattern.[40] The last three of the eight patients had received injections into their glabellas without being informed of the infection risk, which is substantial in an acne-prone facial area like the glabella.[40]

TABLE 1.

Polymer Hydrogels				
Amazingel	Fuhua Medical High Molecular Matter, China	3–5% polyacrylamide Bottles and syringes	Permanent	Yes, in a few countries Banned in China
Aqualift	National Center of Medical Technologies, Ukraine	2–4% polyacrylamide Bottles and syringes	3–5 years	Available on the internet
Argiform	Bioform, Russia	5% polyacrylamide with silver ions Bottles and syringes	Permanent	Yes, in a few countries
Aquamid	Contura International, Denmark	2.5% polyacrylamide Syringes	Permanent	Yes, in over 40 countries outside the US and Canada
Beautical	Rofil Medical, Netherlands Closed 2009	3% polyacrylamide syringes	2–5 years	No longer
Bio-Alcamid	Polymekon research, Italy	3–4% polyakylimide syringes	Permanent	Yes, in a few countries outside Europe and US
Esteform	National Center of Medical Technologies, Ukraine	3% polyacrylamide Bottles and syringes	Permanent	Available on the internet
Eutrophill	Procytech, France Closed 2009	2.5% polyacrylamide syringes	1–5 years	No longer
Evolution	Procytech, France Closed 2009	6% polyvinylhydroxide microspheres in 2.5% polyacrylamide	Permanent	No longer
"Interfaill"	generic manufacture, Ukraine	2–9% polyacrylamide Bottles and syringes	Permanent	Yes, in a few countries Available on the internet
Outline	Procytech, France Closed 2009	3% polyacrylamide syringes	1–5 years	No longer

TABLE 2.

Polyacrylamide Hydrogels			
Elasticity	48–230 Pa	197–418 Pa	33–55 Pa
Acidity (pH)	3.3–4.0	7.4–7.7	7.3–8.2
Dry matter content	3.9–4.9	3.1–3.5	2.2–2.8
Degradation product from polymerization process (Sulphate)	709 ppm	unknown	4 ppm
Injection needle size	14–18 G	14–18 G	25–27 G
Injection technique	Bolus injection	Bolus injection	Multi-line injection
Tissue interaction	Encapsulation	Encapsulation	Fibre and vessel ingrowth

The manual process of withdrawing the hydrogel from a bottle or vial with a syringe rather than a ready-to-use product delivered in a sterile single use syringe increases the risk of contamination. This is probably why late-onset infections or large masses of pus emerging from breasts and buttocks appear up to several years after large bolus injections.[13,17-25,27,37-39] Cells of the immune-regulatory system are unlikely to enter the center of a large implant whereby bacteria will remain undetected. Further, in cases where implants have been encapsulated, cells of the immuno-regulatory system are unlikely to be able to pass the external fibrous capsule surrounding the bolus of hydrogel. Therefore, contaminating bacteria will probably be allowed to grow and multiply undisturbed within the large gel volume for some time, possibly forming a biofilm community before they have reached a number that gives clinical symptoms.[41]

DISCUSSION

While polymer hydrogels for aesthetic use might seem at first glance to be the same material in different packaging, differences become evident on closer inspection. These differences appear in composition, manufacturing processing and control, implantation procedure, and tissue interaction. Each of these parameters has the potential to greatly affect the safety and performance of the product as highlighted by the findings that bolus injection of some polymer fillers may result in more complications than injections of small deposits with other polymer products that allow tissue integration and prevent biofilm formation.[41] Polymer products, including different PAAH products, should therefore not be used interchangeably. The level of evidence for the safety and performance varies greatly from product to product and the lack of safety and performance data for some polymeric hydrogels hinders accurate comparison between products. However, common to them all is the risk of bacterial contamination, and it is of outmost importance always to follow guidelines and avoid conditions that could trigger infection. This means that only PAAH fillers with a proven adequate safety profile should be used under strict regulations for intended use in aesthetic treatments.

> Polymer products, including different PAAH products, should therefore not be used interchangeably.

CONCLUSION

The performance and safety profile of different polymer products for aesthetic use is not only given by their composition. Performance profiles also depend on chemical and physical characteristics including rheological properties, manufacturing process, injection technique, and tissue integration. PAAHs are often considered equal, while having clear differences in composition, manufacturing and injection technique that determine safety and performance of each of these hydrogels. Therefore, equivalent profiles among fillers can only be ensured, if all these characteristics are the same, or if there is scientific and clinical evidence of their similar safety and performance. Only PAAHs with a proven safety profile should be used in aesthetic treatments and only for its intended use.

DISCLOSURES

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