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Original article

Human Histology of a New Bulking Agent Containing PMMA-Microspheres for Gastric Reflux and Urinary or Fecal Incontinence

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Abstract

Background and study design: Bulking agents are currently not available for the treatment of gastroesophageal reflux disease (GERD) and only play a secondary role in the treatment of stress urinary incontinence (SUI) and fecal incontinence (FI). The main reasons for their reluctant use are a relatively high rate of reported adverse events with currently approved materials and competitive technologies. Microspheres made from polymethyl-methacrylate (PMMA) have been successfully injected as dermal fillers for 30 years. They produce a soft and pliable "living implant", do not dislocate, and meet all characteristics of an 'ideal' injectable bulking agent. ***Methods:*** To examine the histological responses to 125µm PMMA microspheres, 28 different blebs were injected subcutaneously into a human forearm and excised after 1 week, and 1, 6, and 12 months. ***Results:*** At 1 month, most spheres were engulfed by a large number of macrophages, which later merged into one giant cell embracing each individual microsphere. At 1 year, most macrophages had left the implant, whose wide interspaces were filled with capillaries and collagen fibers. Monophasic highly cross-linked hyaluronic acid (HA) generates about 65% tissue. In comparison, biphasic HA, which does not keep the heavier PMMA microspheres in suspension, generates around 50% tissue, similar to the carrier material atelo-collagen (AC). ***Conclusions:*** PMMA microspheres produce a soft, fully vascularized "living implant", which is resistant to erosion, migration, and dislocation. The most effective available carrier appears to be monophasic HA-gel, which keeps the beads apart and allows tissue in-growth at about 65% of the total implant volume.

Keywords: ***Human Histology, Bulking Agent, PMMA-Microspheres, Dermal Filler, Gastric Reflux, Urinary Incontinence, Fecal Incontinence***

Introduction

Gastroesophageal reflux disease (GERD) appears to be related to lower esophageal sphincter (LES) incompetence and affects hundreds of millions of people worldwide [1]. Today´s gold standard therapies include daily use of proton pump inhibitors (PPI) or laparoscopic gastric fundoplication [2]. The average long-term success rate is approximately 70%; 10% of the patients have postoperative adverse events; 20% change treatments [3] with only 40% reporting improvement.

Only few injectable 'bulking' agents for GERD, stress urinary incontinence (SUI), and fecal incontinence (FI) have been tested and FDA approved since the early 2000s and several were discontinued for safety concerns. Most bulking agents for FI and SUI were derived from dermal filler materials like polyacrylamide gel (Bulkamid®) [3,4] or dextranomer beads (Solesta®) [5]. Injectable bulking agents for GERD are still in clinical development and not yet FDA-approved for injection augmentation of the LES, e.g. carbon-coated zirconium oxide beads (Durasphere®), or PMMA microspheres (G125). Due to the lack of knowledge of histological reactions and effects of most bulking agents, a human skin histology study was conducted with the aim to answer the following questions [6]. How can a physician inject any dermal filler or bulking agent without knowing what histologic reactions they may cause in the skin or internal organs? What is their mechanism of action, and how about their absorption over time and duration of effectiveness?

Does filler remain at the site of injection, or does it dissipate into the surrounding tissues similar to some fluid fillers like liquid silicone? What kind of cells attack or remove the different types of fillers? Which fillers may induce an excessive

foreign body response or granuloma formation? What is the frequency of adverse events and how can these be managed? How effective are antidotes or enzymes in patients after 'overfilling'? And finally, how effective are intralesional injections of corticosteroids in the treatment of late-onset inflammatory adverse reactions?

Study design

Human histology of polymethylmethacrylate (PMMA) microspheres of 40 µm in diameter has been well documented throughout the 30-years of injectable Artecoll®, Artefill®, and Bellafill® use [7,8,9]. After the use of fluid silicone in the 1970s and bovine collagen in the 1980s, in 1990 injectable PMMA-microspheres became the first dermal filler with a safe profile and long-lasting effectiveness [7]. Injectable PMMA-microspheres were approved in Europe in 1994, in Brazil in 2000, in China in 2002, and in the USA in 2006 [8,9].

For prospective submucosal injections in the esophagus, urethra, and anal canal however, bigger spheres of >100 microns must be used due to larger blood and lymphatic vessels. All three organs are surrounded by venous plexus and vessels of an average diameter of 80 µm. Transportation of 40 µm PMMA to downstream organs including liver and lungs through veins is theoretically possible and must be prevented by the use of PMMA-microspheres of 125 µm or greater in diameter [10,11].

Injections of 40µm-PMMA-microspheres have shown to stimulate the ingrowth of connective tissue and vessels into their implant [12] (**Fig.1**). As a normal 'foreign body' reaction, it is not based on 'rejection' but rather on sealing off and rendering the beads harmless. However, such a host reaction had not yet been documented for injected 125µm PMMA spheres representing a 30X larger volume. Furthermore, histologic evaluation of 40µm-PMMA tissue sections showed that 80% of bovine collagen carrier material was fully replaced by 'living' connective tissue ingrowth during the initial 1-3 months [12].

PMMA-microspheres from different manufacturers worldwide have a slightly irregular surface (**Fig. 2**) which renders them attractive for macrophages to phagocytize these foreign bodies. The same reaction however does not occur with microspheres that have an absolutely smooth surface like carbon coated Durasphere® [13], the gel surface of Embosphere® [14], droplets of fluid silicone, polyacrylamide gel (Bulkamid®) [3,15], or hyaluronic acids (Juvederm®, Restylane®).

These injectables attract none or only few macrophages, and their injected implants are often not sufficiently anchored in the tissue. Interestingly, the rough surface of microspheres from calcium-hydroxyl-apatite (Radiesse®, Coaptite®) [16] also does not attract macrophages. This material is the same as anorganic bone and therefore too biocompatible to be recognized as a foreign body. It is instead broken down by osteoclastic enzymes and then removed by macrophages (see **Fig. 16**).

What percentage of connective tissue will develop between the injected 125µm PMMA in an absorbed atelo-collagen carrier? Might a certain hyaluronic acid material be a better carrier for PMMA microspheres because it will keep them farther apart over months than the rather watery atelo-collagen (AC) or carboxymethyl-cellulose (CMC), both of which are absorbed within the first days after injection? To answer these questions, the following PMMA-suspensions were injected into the skin of a human forearm and the histological reactions were examined.

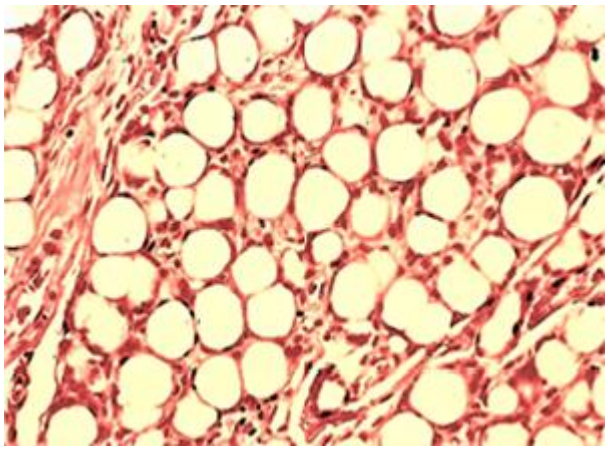

Fig. 1: A 40µm-PMMA implant at 3 months: the invading macrophages and fibroblasts need blood supply and arterioles are seen in the middle left and at the bottom right. It is a "living implant".

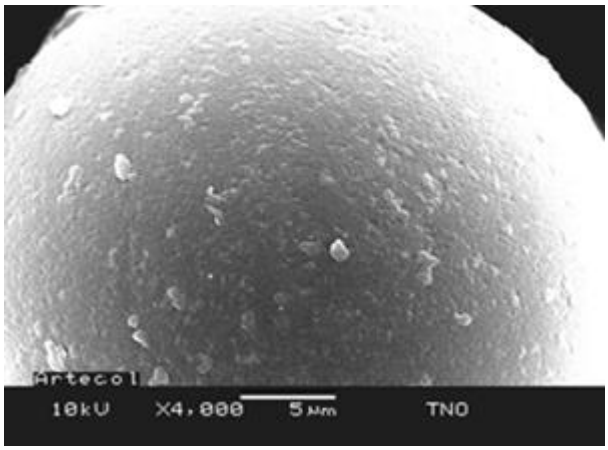

Fig. 2: The secret of injected PMMA-microspheres to attract macrophages are their little irregularities on their hydrophilic surface.

Material and Methods

Sterile PMMA-microspheres of sizes 40µm and 125µm and sterile bovine atelo-collagen (AC), were provided by AscentX Medical, Inc., San Diego, California. A-telo means without allergenic branches, i. e. collagen molecules, which will cause no hypersensitivity or allergy.

The 125µm-PMMA microspheres of Lot# L8.001 and the 40µm PMMA microspheres of Lot# AO901 were examined for size distribution, uniformity, and purity by Microparticles GmbH, Berlin, Germany.

Biphasic hyaluronic acid (HA) gels consist of cross-linked particles suspended in a stabilized gel of 24mg/ml HA (Amalian® expert III from Fa. Skin-Vision, Heringsdorf, Germany). A special technology makes this product easy to inject in spite of its high structural viscosity.

Monophasic HA gel of 23 mg/ml HA, which is cross-linked to a great extent (Teosyal® RHT4 from Teoxane Laboratories, Geneva, Switzerland) served also as a suspension medium for the 125µm-PMMA powder. Both PMMA powders were brough into suspension with both HA gels with the help of a sterile syringe connector.

1. 10 % 125µm-PMMA suspended in 3.5% AscentX-AC
2. 20 % 125µm-PMMA suspended in 3.5% AscentX-AC
3. 30 % 125µm-PMMA suspended in 3.5% AscentX-AC

4. 20 % 125µm-PMMA suspended in 2.4% Amalian (biphasic HA)
5. 20 % 40µm-PMMA suspended in 2.4% Amalian (biphasic HA)
6. 20 % 125µm-PMMA suspended in 2.3%Teosyal (monophasic HA)

The subject is a healthy 84-year-old plastic surgeon who has been testing histologic responses to all different dermal fillers in his forearm for 30 years [7,16]. He injected the content of the 6 syringes subdermally into his forearm in form of 0.1 ml blebs (**Fig.3**), the 40µm-PMMA through a 1/2"x 25G needle, the 125µm-PMMA through a 1"x 23G needle. The blebs were numbered and documented with photographs and their distance was measured from certain landmarks of the skin (scars and age spots). Surgical excisions of full-thickness skin samples for histological examination took place after 1 week, and 1, 6, and 12 months.

Human skin of a volunteer forearm allows for faster testing compared to animal skin, because it does not require review and approval by an Institutional Review Board (IRB) and ethics committee. There are no ethical or legal barriers to harmless self-experimentation [17], and the filler and bulking agent industries will benefit from consecutive and long-term human histology at various time points.

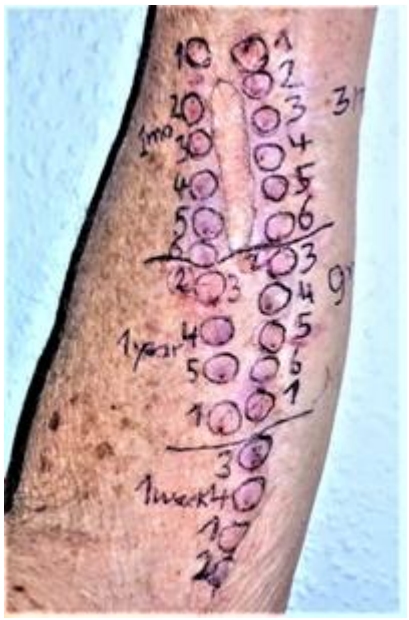

Fig. 3: Five strips of 6 injected blebs of PMMA-microspheres in different carriers and concentrations are waiting for excisions after 1 week and 1, 6 and 12 months.

Results

a) Three different concentrations of PMMA-microspheres suspended in atelo-collagen (AC)

At one week, the watery carrier AC was absorbed in all specimen with 10%, 20%, or 30% 125µm-PMMA by volume and all microspheres were sticking closely together (**Fig. 4a**). The invading macrophages had tried to engulf them but merged to giant cells, which kept the spheres firmly anchored in the implant (**Fig. 4b**). At 6 months, fibroblast had started to produce a network of inter-weaving collagen fibers (**Fig. 4c**); neo-vascularization appeared to be a prerequisite (**Fig. 1**). At 1 year, most macrophages and fibroblasts had left the implant and allowed connective tissue to fill the interspaces (**Fig. 4d**). This histologic outcome will be permanent. (**Fig. 5**).

No histological differences were found between the 3 different concentrations of 125µm-PMMA microspheres. The size of the each developing dermal bleb was difficult to estimate from two-dimensional histology sections. One may assume however, that a syringe filled with 30% PMMA will produce a mature and remodeled implant 1/3 bigger than a syringe with 20% PMMA content. If a more viscous bulking agent with 30% PMMA will be injectable through a 135 cm long catheter as used with standard gastroscopes as easily as a product with 20% PMMA, esophageal bulking may occur faster, more efficiently, and become more cost-effective.

b) Suspension in biphasic HA-gel containing crosslinked particles

It appears to be easier to keep cross-linked HA-particles in suspension in an HA-solution compared to heavier PMMA-spheres with a molecular weight of 180,000 g/mol. After one week, 40µm or 125µm microspheres were lined up on the inner shell of the biphasic implant, probably due to gravitational forces (**Fig. 6a**). Over the months, this shell of fibers, cells, and spheres prevented hyaluronidase contained in the soft tissue from breaking down the inner HA-gel and preserved most of the injected volume over more than a year (**Fig. 6b**)

c) Suspension in extended cross-linked monophasic HA-gel

How long would the monophasic HA-gel keep the microspheres in suspension before all were encapsulated and surrounded by connective tissue? At one week, all injected microspheres were covered with fibrin and macrophages had invaded only the two outer rows of microspheres. During the following months (**Fig.7**), the microspheres were kept apart to allow further invasion of cells to cover the beads (**Fig. 8**). At 6 months, most of the HA was absorbed but still detectable; and at 1 year, the single beads were much further apart (**Fig. 9b**) than in the 125µm-PMMA in AC specimen (**Fig. 8**)

d) The amount of induced autologous tissue after 1 year:

In order to document how many cells and collagen fibers were induced by the bigger 125µm beads, 5 histological sections were printed on DIN A4 paper. The diameters of the white areas of the contained 125µm spheres were measured as r2. Their total area was subtracted from the total area of the histological section. The deduction of the areas of spheres on five sections of 125µm-PMMA in AC resulted in a mean value of 52.43% (39.3% to 63.9%) connective tissue filling the interspaces of the spheres. The results revealed that 125µm PMMA spheres stimulated cells and collagen fibers to about 50% of their own volume. (**Fig.4d**).

On five sections of 125µm PMMA suspended in biphasic HA at 1 year, the deduction of white sphere areas from the sheet revealed an average of 47.7% (37.2%-54.2%) tissue developed around the beads. These numbers resemble those of 125µm-spheres in AC. In contrast, the measurements on five histological sections of 125µm-PMMA suspended in monophasic HA revealed at 1 year an average of 64.9% (54.3%- 73.3%) new collagen and tissue had formed around the 125µm spheres (**Fig.9b**).

These results allow the conclusion, that monophasic highly cross-linked-HA with 65% tissue bio-stimulation will be the optimal future carriers for permanent injectable bulking agents or dermal fillers. Commercially available sterile HA is more cost-effective than bovine AC solution, which is a rather costly extract obtained from BSE-free calf hides from 'closed' herds and multiple chemical viral inactivation steps.

PMMA microspheres are firm and solid, while connective tissue is soft and pliable. The greater the percentage of pliable

autologous fibro-vascular tissue in a fully remodelled filler or bulking agent, the more biocompatibility and safety can be expected, considering the risk of inflammation, erosion (sloughing), perforation, or dislocation as observed with discontinued materials [6].

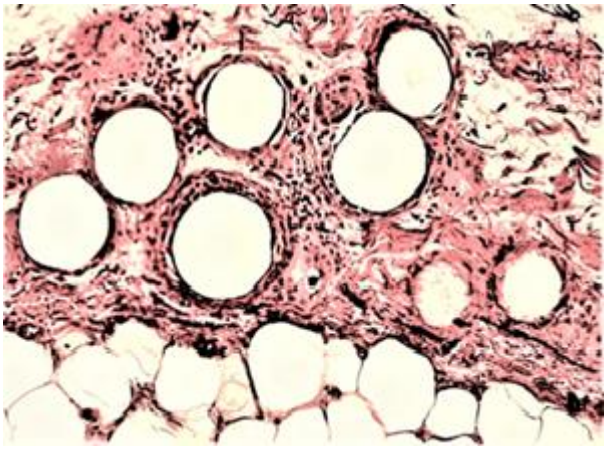

Fig. 4a: At 1 week, macrophages and fibroblasts are invading the first 3 rows of 125µm microspheres in AC and fix them exactly where they were injected.

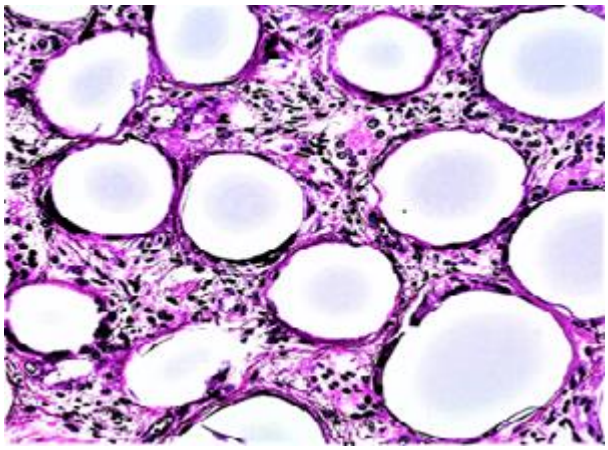

Fig. 4b: At 1 month, a high number of macrophages of 10 µm in size are attached to 125µm-PMMA microspheres. Fibroblasts are filling the interspaces.

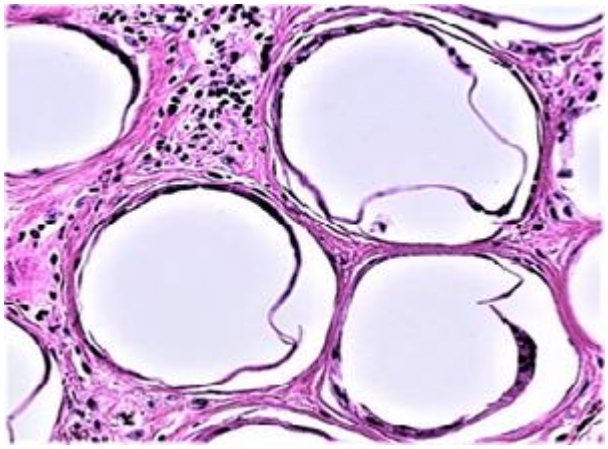

Fig. 4c: At 6 months, all macrophages have merged to form one huge giant cell to hold one single G125 microsphere in position. Collagen fibers are filling the interspaces and hold the implant in place.

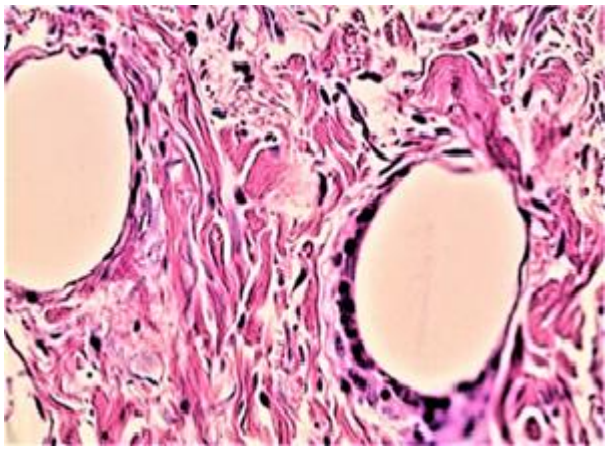

Fig. 4d: At 1 year, the implant shows how only one single giant cell embraces one huge 125µm PMMA-microspheres. Most macrophages and fibroblasts have disappeared, and collagen fibers are filling the interspaces.

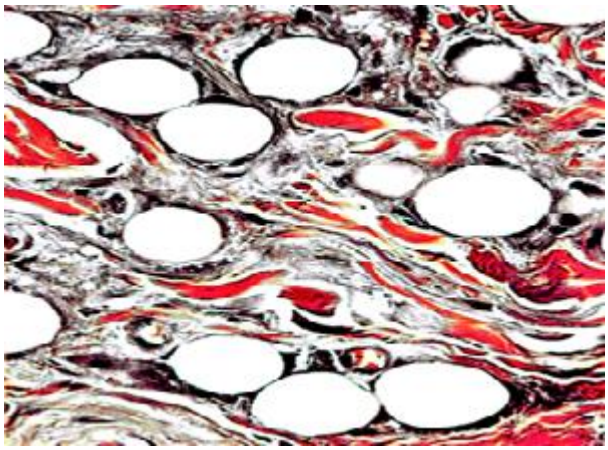

Fig. 5: 10 years after the injection of PMMA-microspheres are fibroblasts producing broad bands of collagen (red) and single giant cells are still embracing one microsphere.

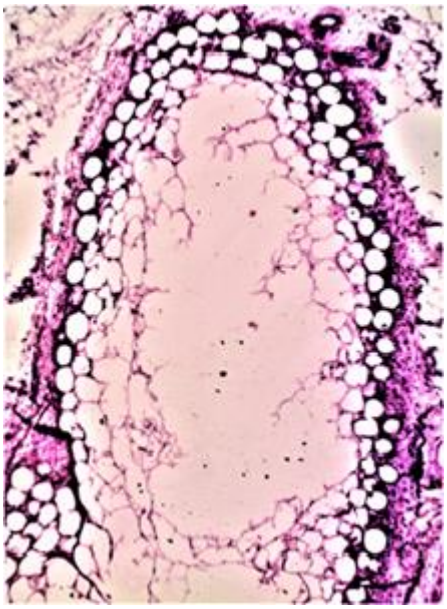

Fig. 6a: At 1 month, all microspheres in biphasic HA have separated from their carrier and are sticking to the inner wall of the HA-bleb where macrophages are invading the first 3 rows of beads.

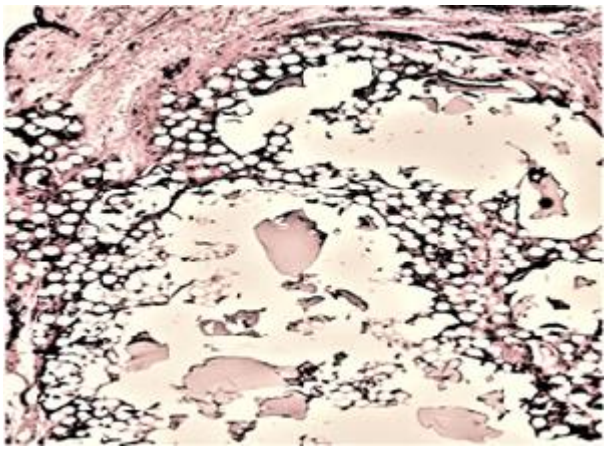

Fig. 6b: At 1 year in biphasic HA, all microspheres are covered by macrophages, however, most HA is not yet degraded by hydrolytic enzymes.

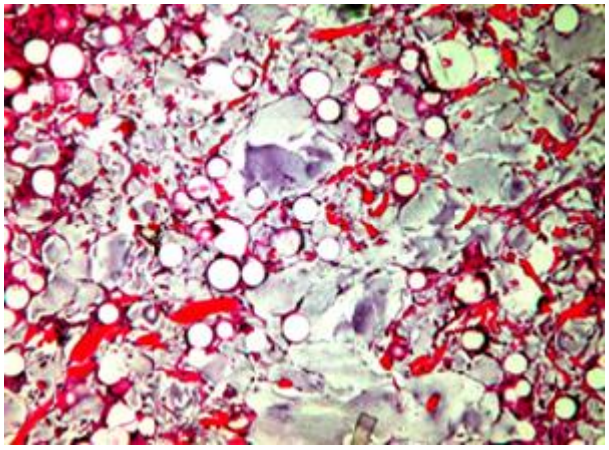

Fig. 7: At 1 month, the monophasic HA is keeping the microspheres apart and allows more space for invading macrophages to cover the scattered microspheres.

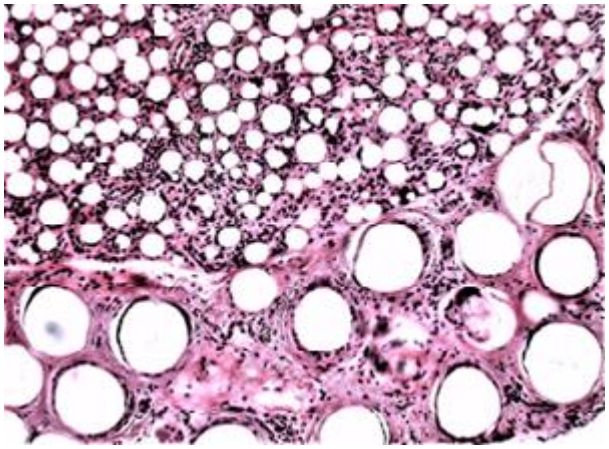

Fig. 8: At 6 months coincidentally, 40µm PMMA and 125µm PMMA microspheres in biphasic HA were injected side by side. The smaller ones stimulate about 80% tissue, the larger ones only about 5o%.

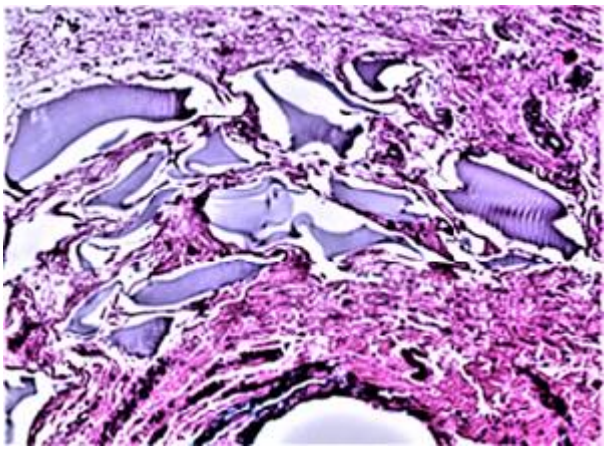

Fig. 9a: At 6 months, residues of monophasic HA (blue) are detected outside the 125µm-microspheres.

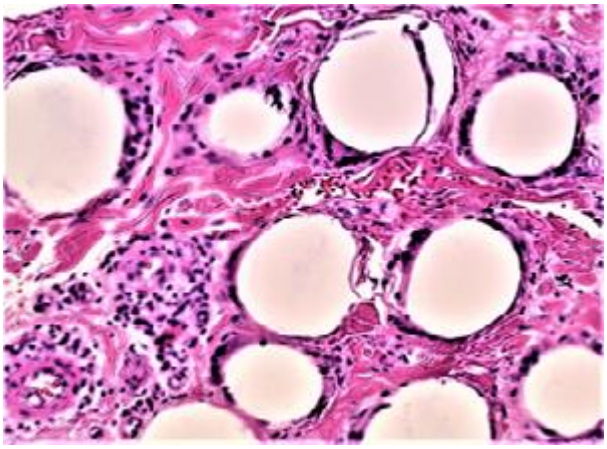

Fig. 9b: At 1 year, the monophasic HA is largely absorbed but left enough space for 66% tissue as filler substance between the 125µm microspheres.

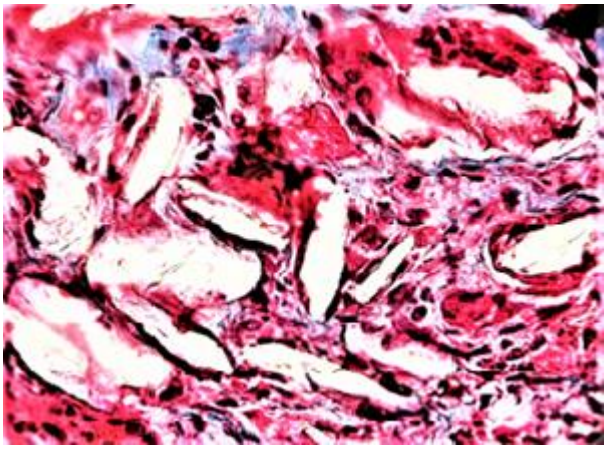

Fig. 10: At 1 year, Coaptite microspheres are slowly degraded by enzymes and phagocytized by macrophages converted to osteoclasts. No fibroblasts and collagen fibers are detectable.

Discussion

For the past 40 years, tissue biocompatibility and persistence of various filler materials has been of great interest in dermal filler research. Finding the most suitable carrier material for micro-particulate fillers, whether bovine AC, HA, or CMC, will be an important issue in developing superior bulking agents in the future.

Human dermis remains an ideal tissue for determining the optimal concentration of microspheres in fillers and bulking agents to test injectability through longer catheters if required. Our

specifically developed injection systems for GERD and SUI include 23G needles and safety stoppers to prevent transmural implant placement. PMMA-microspheres are the only permanent injectable material that stimulates enough autologous connective tissue to create a "neo-vascularized bulking agent" or "living implant" and induce "lasting neo-collagenesis" [7,16].

Study Limitations

Macrophages attack injected foreign bodies the same way after subcutaneous injection as in submucosal injections in esophagus and in urethra, and form a "living implant", surrounded with a fibrous capsule. The unknown is rather the looseness of the submucosal layer in esophagus and urethra or anus, i.e. whether the implant will remain sufficiently fixed at the site of injection for years? In the lower esophagus it is injected above the gastric entrance, which stops it from slipping downwards by its fibrous ring. In the urethra and anus, there is no such ring.

However, our previous experiments on pigs [10,11] proved that the same implants in the esophagus and urethra were still fixed at the injection site after 90 days, i.e. they did not sag. This could happen in humans with loose connective tissue in the urethra and anus, as heavier bulking agents (Durasphere) have already shown.

Comparison with available bulking agents

Dermal fillers and bulking agents are differentiated as particulate or non-particulate [18]. Particulate ones exert their effect by stimulating connective or granulation tissue to encapsulate every single bead or particle. Non-particulate ones are biocompatible injectable gels, which are encapsulated by a fine network of fibers; they stimulate small amounts of connective tissue but work as "volumizers" until they are absorbed.

a) *Contigen*® (C.R. Bard, Murray Hill, New Providence, NJ)) was the first injectable bulking agent for SUI and consisted of cross-linked bovine collagen. It was discontinued in 2011 due to increasing competition by longer-lasting materials When used off-label for GERD, large amounts of 30mL were injected to create a 'mucosal plug' at the lower esophageal sphincter because most of the injected material was erroneously placed extramural. The injected collagen was quickly absorbed by the body, and due to its short tissue persistence, proved unsuitable as an esophageal bulking agent [19]. In 1985, PMMA-microspheres were added to the available bovine collagen implants (Collagen Corporation, Freemont, California) to prolong longevity in wrinkle treatment [7].

b) *Macroplastique*® (Laborie Medical Technologies Corp., Portsmouth NH) contains solid silicone flakes of 200-600µm in size, suspended in a polyvinyl-pyrrolidine (PVP) carrier gel. It was FDA-approved as urinary bulking agent (UBA) in 2006 [20]. There are no known reports of Macroplastique off-label use in GERD patients. As dermal filler, Macroplastique caused a high rate of foreign body granulomas due to its irregular surface [21] and smaller particles were transported to lungs and liver, before it was taken off the dermal filler market.

c) *Durasphere*® (Carbon Medical Technologies, Inc., St. Paul, MN) consists of heavy zirconium oxide beads which vary in size between 250-300µm. They are suspended in an aqueous gel of 2.8% ß-glucan. Their surface is covered with non-biodegradable pyrolytic carbon for absolute smoothness. Inflammatory reactions have been described as mild [21], however heavy zirconium oxide beads have been known to descend by gravity in patients with looser connective tissue [22]. In 1999, Durasphere received FDA market approval for stress urinary incontinence, but has not gained wide market adoption.

d) *Coaptite*™ (Boston Scientific Corp. Marlborough, MA) [4] contains microspheres of calcium hydroxylapatite (CaHA) of 75-125µm in diameter suspended in a gel of sodium carboxymethyl-cellulose (NaCMC). Coaptite received the CE-mark in 2001 and FDA-approval in 2005 [4,18]. Calcium hydroxylapatite is found in human bones and teeth and is therefore completely biocompatible, but lacks effectiveness in bio-stimulating autologous connective tissue [16]. It is degraded by osteoclastic enzymes (**Fig. 10**) and has shown only minor foreign body rection [21].

e) *Bulkamid*® (Axonics Modulation Technologies, Inc., Irvine, CA) is a clear gel of 2.5 % polyacrylamide gel (PAAG) in water. It is highly biocompatible and following periurethral injections, it is anchored in situ within a fine network of collagen fibers [18]. Bulkamid has been used world-wide outside of the United States since 2003 for aesthetic dermal indications (as Aquamid®) and most recently has received FDA approval in 2020 as a urinary bulking agent (UBA) to treat SU I [3,4]. Polyacrylamide is slowly absorbed after enzymatic destruction [16].

f) *Deflux*® (Palette Life Sciences, Santa Barbara, CA), a dextranomer hyaluronic acid (DxHA) copolymer, is an injectable material with proven efficacy in vesico-ureteral reflux. It also received FDA approval as an injectable bulking agent for gastro-urology use in 2005. It consists of 50mg/ml dextran-microspheres of sizes between 80-250 µm, suspended in 15 mg/ml HA.

To date, Deflux has been used in children with ureteral reflux and in one trial on GERD patients [15]. Dextran beads may cause a strong foreign body reaction, where one single bead is engulfed by one huge giant cell. Several reports on late calcification of Deflux implants called for caution. In 2011, the same material under the tradename Solesta® (Oceana Therapeutics, Edison, NJ) was FDA approved as the first and only anal bulking agent (ABA) for the treatment of fecal incontinence.

Possible adverse events after injection of bulking agents

a) *Transportation of particles* from the injection site to distant organs, such as liver, lungs, spleen, or lymph nodes, has only been described for small particles <40 µm [22,23].

b) *Embolism* and local tissue necrosis are well-known phenomena after intravascular injections of dermal fillers where the needle tip penetrates an artery or vein during injection. Blunt needles and constant moving of the needle back and forth during injection may prevent such serious adverse events that can cause skin necrosis and blindness and have been experienced with all filler materials injected in the face.

c) *Dislocations* of entire implants after injections of bulking agents have rarely been described [24,25] but may occur in patients with extremely loose connective tissue.
d) *Erosions and perforations* of solid and firm implants have been rarely experienced [26] after insertion into constantly moving muscle tubes, such as esophagus, urethra or anus.
e) *Immune reactions* are known to occur with all fillers, especially those containing particles or droplets up to the size of macrophages (10-20 µm). Sudden generalized bacterial infection can cause cellular hyperimmune reactions years after filler injections [21]. Granulomas or late-onset inflammatory adverse reactions (LOIAR) have been described after injections into sensitive skin only, but not after injections of bulking agents.
f) *Short-term efficacy* of UBAs is generally encouraging, however longer-term follow-up studies show decreased success rates and patients may require repeat treatments [26,27]. Available data comparing currently available UBAs with sling procedures, such as tension free vaginal tape (TVT), demonstrate poorer outcomes for injection therapies than for surgery [28]. PMMA microspheres, however, are firmly anchored where injected and maintain their permanent bulking effect. For this reason, once FDA approved, they have the potential to evolve into the preferred bulking agent for the treatment of GERD, SUI, and FI.

Still hypothetical are stem cell treatments, which are being developed to functionally regenerate sphincter muscles in patients with sphincter deficiency. Autologous pre-adipocytes and muscle-derived stem cells may become the preferred stem cell types, as they can be easily harvested and cause minimal donor site morbidity [28].

Conclusions from histology

1. At one week, the AC-carrier had been fully absorbed and the beads were sticking together. A similar picture occurred in the specimen with the biphasic HA-carrier, which caused the 40µm and 125µm spheres to sink and clump along the inner wall of the HA-blebs.
2. At 1 week, macrophages from the surrounding tissue invaded the first 2-3 rows of the microspheres and began to phagocytize, i.e. to encapsulate and fix them.
3. At 1 month, all single 40µm-PMMA spheres were engulfed by a few smaller 10µm-macrophages. The 30x bigger 125µm-PMMA spheres were covered by approximately 20 macrophages, which later merged to a single giant cell embracing each individual microsphere.
4. At 12 months, biphasic HA-implants still contained a large amount of carrier HA. Apparently, the tight wall of fibroblasts prevented hyaluronidases from entering the HA-bleb and dissolving the HA.
5. Different concentrations of 10%, 20% or 30% 125-PMMA spheres provided a similar histologic image: The early absorption of AC and the separation of biphasic HA carrier led to the development of only 50% tissue and 50% spheres.
6. After early absorption of the AC-carrier, an injectable with 30% PMMA spheres will cause an implant 1/3 bigger in size than a 20% PMMA injectable, which would also be more cost-effective.
7. PMMA 125µm spheres suspended in monophasic HA-gel stimulated approximately 65% tissue, which filled the interspaces of 35 % PMMA spheres. PMMA microspheres suspended in biphasic HA or AC stimulated only 50% tissue.
8. A higher percentage of tissue creates a more pliable implant, which prevents inflammation, erosion, and perforation, and guarantees longevity inside a constantly moving muscular tube, such as the esophagus, urethra, and anal canal.
9. At present, the most effective carrier for microspheres appears to be monophasic HA-gel, which keeps the spheres apart and allows for more tissue ingrowth.

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Conflict of Interest

The author has no conflict of interest

Disclosure

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