Research article

The role of biomaterials in upper digestive tract transoral reconstruction.

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Abstract: Background: This study aims to establish whether the use of biomaterials, particularly polydimethylsiloxane (PDMS), for surgical reconstruction of the esophagus with templates, Montgomery salivary tube, after radical oncology surgery for malignant neoplasia is an optimal choice for patients' safety and for optimal function preservation and organ rehabilitation. Methods: Structural analysis by Raman spectrometry and biomechanical properties with dynamic mechanical analysis are performed for fatigue strength and toughness, essential factors in durability of a prosthesis in the reconstruction practice of the esophagus. Nanocomposites with silicone elastomers and nanoparticles used in implantable devices and in the reconstruction surgery are facing risks of infection and fatigue strength when required to perform a mechanical effort for long periods of time. Results: This report takes into account the effect of silver (Ag) nanoparticles on the fatigue strength using polydimethylsiloxane (PDMS) matrix, representative for silicon elastomers used in implantable devices. PDMS with 5% (wt) Ag nanoparticles of 100-150 nm during mechanical fatigue testing at shear strength loses elasticity properties after 400 loading-unloading cycles and up to 15% shear strain. The fatigue strength, toughness, maximum shear strength are the key issues in designing Montgomery salivary tube with appropriate biomechanical behavior for each patient. Conclusions: Prosthesis design needs to indulge both clinical outcome as well as design methods and research in the field of biomaterials.

Keywords: malignant neoplasia, transoral reconstruction, polydimethyl siloxane, Ag nanoparticles, fatigue strenght, prosthesis

1. Introduction

Silicone is used for head and neck implantable devices in the form of cochlear implants [1], nose implants [2] and prosthesis of the upper respiratory and digestive tract [3,4].

The main issue of implantable prosthesis is by far related to the physical and mechanical characteristics regarding strength, shear strain and longevity. For this issue to be addressed some improvements in the structure of the silicone polymers used have been made. Nanocomposites are the result of different molecules incorporated in the structure of the polymers [5,6] The development of such composite mixtures must increase the performance, utility, productivity and product uniformity [7,8], as well as to ensure optimal blend by polymer dispersion for improved properties [9].

Depending on the polymers used to increase the strength and shear strain different results can be achieved. In the head and neck region a silicone elastomer mixed with nano alumina ceramic fiber was used by Nouri Al-qenae for facial reconstruction. However, their results established that this mixture does not have significant outcome in terms of needed properties [10]. Nonetheless, Sara M. Zayed et al. and Dhuha A. Shakir and Faiza M. Abdul-Ameer conducted a series of mechanical testing regarding SiO2 nanoparticles, respectively TiO2 nanoparticles. Results showed that after incorporation all mechanical properties of the polymers improved [11,12].

More recent studies concerning silicone polymers revealed that 3D printed prosthesis is likely to suffice mechanical problems like viscosity when compared to the classical indirect molding technique, as described by Eric et al. [13].

Viscosity of the polymer tends to act inversely to strain hardening effect in terms of mechanical cycles, therefore less viscosity more cycles [14].

Malignant neoplasia of the upper digestive tract is subject to oncology therapy which implies surgery. In most cases, radical resection needs to be performed and the tissue defect needs to be replaced so that function can be restored. Several techniques have been imagined and used in this type of reconstruction. In the E.N.T. Clinic of Colțea Clinical Hospital, we developed a proprietary method which resides in the use of a prosthesis by which the upper digestive tract continuity is being re-established.

Biomaterials used for reconstruction after oncology reconstruction must fulfill demands of biocompatibility ranging from simple biomechanical use to high bioaffinity with tissues and organs. In general, biocompatibility refers to implantable medical devices and organ replacements for reconstructive surgery. Multiple reviews and research focus on different types of biomaterials with specific relation to a targeted application. Hierarchically, the biomaterials must perform a main function (or more) with associated biocompatibility defined by local bioenvironment [15-17]. Esophagus reconstruction surgery has a bioenvironment that is quite complex. The biomaterials used in reconstruction are in direct contact with tissues and local organs respective, food (bolus), beverages and saliva, whilst being in indirect contact with external environment. In such applications, they should fulfill several properties:

1. Biomechanical properties of the artificial esophagus to mimic multiple mechanisms by which the esophagus responds to the various physiological demands. The esophagus may have the capacity to change its propulsive force in response to bolus size and neurohumoral agents [18]. In this respect the first mechanical properties are related to the stress-strain mechanical response. The storage modulus needs to be well-matched with the appropriate biomechanics of the patient and the dynamic of the propulsive forces to bolus size.

2. Tribological properties need to be concurrent with the inner surface of the prosthesis with self-cleaning and to have hydrophobic compatibility with saliva and bolus dynamics.

3. The prosthesis needs to have appropriate thermo-physical properties such as low swelling related to the biological fluids, respective water-uptake and need to be chemically inert and non-biodegradable.

4. The prosthesis inner lining needs to have bacteriostatic and antifungal properties.

5. The biocompatibility of the prosthesis needs to apply to the surrounding tissues and to have good connectivity to other organs.

6. The prosthesis needs to be responsive to neurohumoral agents in terms of electroactivity.

Today, the most used templates for esophageal prosthesis are Montgomery salivary bypass tube [19-21], a viable alternative in reconstruction. In the tubular region, the prosthesis has two spherical zones (areas) which assure a better stability and optimal saliva leaking along of tube, independent of head and neck position (Figure 1).
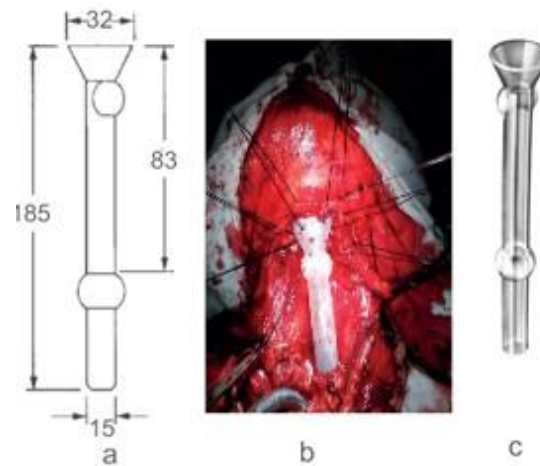
Figure 1.   a) Montgomery salivary tube, constructive principle (dimensions in mm) b) A case study implemented by the Colțea Hospital ENT Team (iconography Dr. C.R Popescu [22]) c) Montgomery tube made of silicon rubber.

In the range of silicon rubbers, polydimethylsiloxanes (PDMS) have several advantages in designing esophageal prosthesis: it can be molded in different shapes in vacuum or by centrifugation for degassing; the ratio silicon resins/hardener (curing agent) can be adjusted to elaborate a cross-linked rubber with convenient biomechanical properties: molecular weight, viscosity, elasticity related to the shear forces; the PDMS surfaces can be treated (such as plasma treatment) to obtain superhydrophobic/hydrophilic properties or to insert various antioxidants and bacteriostatic agents (addition of Ag). In addition, by designing appropriate composites with different nanomaterials PDMS can be radio-opaque or radio-transparent for locally induced radiotherapy. Moreover, it can be designed for appropriate insertion of biopolymers containing various drugs with controlled release for local therapy.

One key issue with PDMS is biomechanical stability during working conditions, such as fatigue strength, maximum strain deformation, storage and dissipation modulus. These characteristics should be accommodated with the biomechanical properties of each patients' tissue particularities where one prosthesis replaces one organ.

This study reviews the mechanical behavior during cyclic loading-unloading by simulating the bolus dynamics (fatigue strength, toughness, shear strain). The samples used to extrapolate end-point data were silicon rubber from Montgomery salivary tube, silicon rubber prepared after appropriate recipes and composites silicon rubber-silver nanoparticles.

The results are useful in surgical reconstruction aiming to restore oral nutrition by transoral prosthesis implantation in the hypopharynx and cervical esophagus. We should mention that cancer of the hypopharynx and cervical esophagus is often diagnosed in late stages due to discrete symptoms, thus being a major health problem related to patient survival rate and implications in quality of life. Surgical reconstruction after radical resection is in most cases a challenge for the oncology head and neck surgeon [22-25].

2. Materials and Methods

Our design for the study encompassed: preparation of PDMS samples for testing, reference and silicone-silver nanoparticle polymers; Raman spectrometry to determine the chemical structure of the polymer; mechanical analysis of the samples and comparison analysis; in vitro testing of prosthesis for biocompatibility and clinical surgery.

2.1. Sample preparation

We designed a study to compare the use of biomaterials in hypopharynx and cervical esophagus transoral reconstruction, with or without mandible reconstruction. For this we used a reference sample, transparent Montgomery esophageal tube (Boston Medical Product, Inc) and PDMS recipes,

described as following. There is a large class of siloxane base oligomers and associated curing agents. Similar to silicon rubber used in Montgomery salivary tube (Boston medical products, Inc) there are series of other silicon rubber used in designing components for microfluidics and soft lithography such as Sylgard 182-186 (Dow Corning).

After curing, the elastomers are translucent and have various mechanical properties following smart design with appropriate ratio silicon oligomers/curing agent. Usually, the recommended ratio is 10: 1 and the curing temperature up to 150^0 C. Slight variations in silicon oligomers/curing agent ratio means that the mechanical properties are well reproduced as Montgomery salivary tube. Samples were tested in the shear stress conditions.

The S1 sample was taken from Montgomery Salivary tube (as specified in figure 1). S1a- samples were prepared from Sylgard 184 to match the resistance properties of the S1 sample and similar mechanical properties. S2-samples were prepared with Sylgard 184 with a specific ratio oligomer/curing agent (10:1) to obtain similar mechanical properties as S1. S2 was prepared by mixing the Sylgard 184 oligomer with silver nanopowder (TEM diameter ~100-150 nm, average hydrodynamic diameter ~230 nm, measured by DLS) in 5% (wt/wt) and then adding curing agent and treated at 130^0C for 30 min. Silver nanoparticles were prepared using specific methods [12]. All samples were shaped so they could form test specimens of 10 mm diameter and 1mm thickness.

2.2. *Raman spectrometry*

Raman spectrometry used for data spectral data analysis included Jasco, NRS-3100 with dual laser beams, 532 and 785 nm, resolution 4 cm^{-1}, with specific configuration and backscattering.

Raman analysis is performed to acquire data from a chemical point of view. We performed the spectral analysis to quantify the number of repeatable unit in PDMS–rubber, to establish PDMS network with cross-linkage on -Si-O-Si- and to verify cross-linked PDMS-network via vinyl groups.

2.3. *Mechanical analysis*:

Mechanical analysis was performed using DMA/SDTA861, STAR SYSTEM, produced by Mettler Toledo. The operating mode implied Shear function (shear force vs shear strain). This mode is ideal for elastomers, thermoplastics and thermosets. In the shear mode, two identical samples were clamped symmetrically between two fixed outer parts (2) and a central moving part, (1) (Figure 2). The shear clamp guarantees a homogeneous temperature distribution. A thermocouple mounted directly in the clamp measures the samples' temperature so precisely that simultaneous heat flow effects could also be determined (SDTA). The shear force F ranged from 1 mN to 40 N, frequency ranged from 1mHz to 1000Hz. The sample diameter had a maximum ≤14 mm and the thickness had a maximum ≤6.5 mm. The experimental set-up included a sample of 10 mm in diameter, thickness of 1 mm, with 1Hz frequency, at room temperature. Maximum shear strain for the samples investigated were 100 microns, respective 150 microns. That corresponded to the increase in diameter of the Montgomery tube at around of 10%, respective 15%.
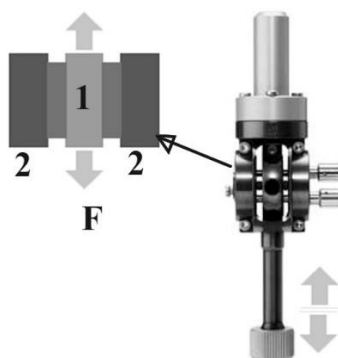

Figure 2. Configuration for shear operating mode in DMA/SDTA 861, STAR SYSTEM, produced by Mettler Toledo. 1-mobile plate, 2-fixed back support. F- shear force.

2.4. *Clinical surgery*

After in vitro testing of the new biocompatible prosthesis we performed clinical testing.

Colțea ENT Clinic used an original implantation technique of the Montgomery prosthesis that met the criteria for optimal reconstruction: refueling facility, oral method, reduced complications and mortality, period of hospitalization with lower costs [22]. In selected cases were mandible reconstruction was needed a multidisciplinary team of general surgeon and orthopedic surgeon were required [27,28].

Esophagus prosthesis used for upper digestive tract reconstruction are prone to fungi colonization, in most cases with Candida albicans. One of our aims during this study was to establish whether silicone elastomers mixed with silver nanoparticles have good biocompatibility and present antifungal properties.

Along with the technique used to surgically implant the prosthesis a concurrent well-known problem appeared. Biofilm formation was observed in sample prosthesis. Infections resulting from microbial adhesion to medical surfaces have been observed on all prosthetic devices, regardless of the biomaterial nature, with severe economic and medical consequences. Biofilm-associated infections raise some clinical challenges, including disease, chronic inflammation, and rapidly acquired antibiotic resistance (Rodrigues, 2011). The biofilm represents a sessile microbial community comprising microbial cells with altered phenotype, characterized by reduced growth rate and altered gene expression [29]. Biofilms are generally included in a protective polysaccharide matrix secreted by biofilm cells and are found attached to a surface [30-35].

The microscopic examination of Gram-stained smears performed directly from the biofilm developed on the surface of the voice prosthesis revealed the presence of a frequent association between different morphological types, indicating the specific nature of microbial biofilm (Figure 3, Figure 4). The examination of the colonized esophageal prosthesis specimens by SEM revealed the presence of a mature biofilm, with a consistent matrix and a complex three-dimensional structure (Figure 5). The microscopic examination of different regions of the prosthetic device has shown that biofilm deposits are accumulating along the entire surface of the device. The well-developed biofilm could be therefore responsible of the prosthesis dysfunction and limited resistance over time.
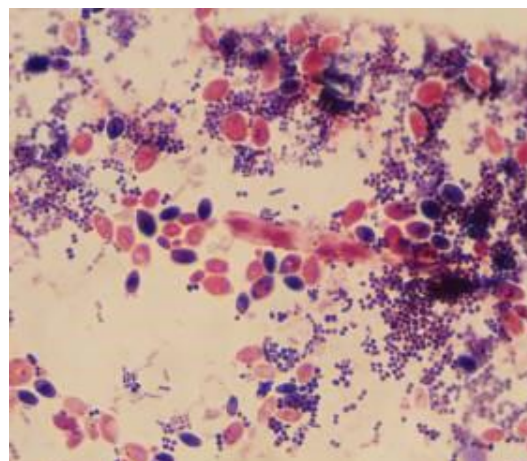

Figure 3. Microscopic images of Gram-stained smears performed directly from the biofilm developed on the surface of the voice prosthesis (Gram stain, x1000).

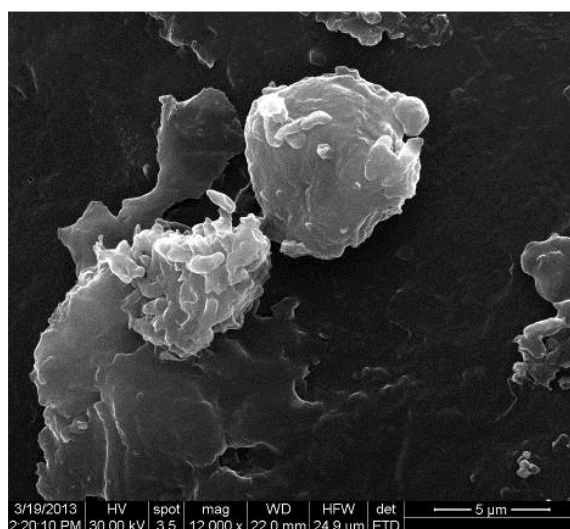**Figure 4.** Biofilm forming on the inner surface of the prosthesis.

Figure 5. SEM images of microbial biofilm developed on the surface of the voice prosthesis.

3. Results

3.1. Raman spectroscopy.

Raman spectrometry used for data spectral data analysis included Jasco, NRS-3100 with dual laser beams, 532 and 785 nm, resolution 4 cm^{-1}, with specific configuration and backscattering. Raman spectra for samples S1 and S1a show specific features for PDMS-rubber. For exemplification spectrum is recorded for S1 (Figure 6).

The spectrum shows several features specific for simple repeatable units (Figure 6a). We identified several bands assigned to –CH$_3$ groups respective to the backbone –Si-O-Si-. They are well defined and have high intensity. The other bands with weak intensity are associated with a particular type of cross-linkage developed in PDMS network: 1585 cm^{-1} (C=C, in phase- stretching), 1442 cm^{-1} (δ(CH2) scissoring), 1377 cm^{-1} (-CH$_3$, methyl rocking), 1146 cm^{-1} (C-C stretching), 1079 cm^{-1} (in-plan rocking for C=CH group). In conclusion, the polymer develops a network with cross-linkage on -Si-O- (Figure 6b) with low levels of cross-linking on –CH$_2$-CH$_2$- vinyl group (Figure 6c).
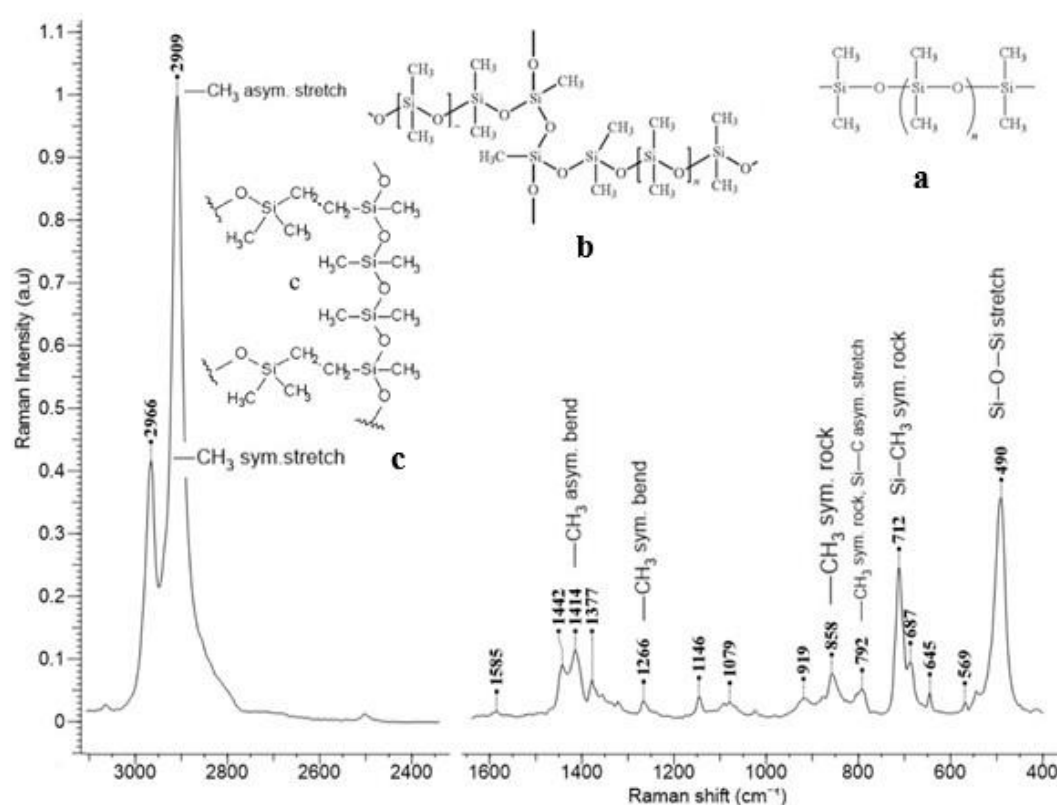
Figure 6.   Raman spectrum recorded for S1, a) the repeatable unit in PDMS–rubber b) PDMS network with cross-linkage on -Si-O-Si- c) PDMS-network cross-linked via vinyl groups.

3.2. Shear stress/shear strain

Mechanical analysis was performed using DMA/SDTA861, STAR SYSTEM, produced by Mettler Toledo. The mechanical properties measured in shear mode are shown in Figure 7 for samples S1 and S1a. For the same reason we show results for S1.

The shear stress/shear strain curve has a quasilinear behavior up to 30 microns per millimeter reaching a plateau at shear strain ~ 100 microns (Figure 6, left). This is consistent with rubber-elastic materials and their capacity to reach a "rubbery plateau" with quasi-reversible return in an initial state [38]. The shear modulus (Figure 7, left) is dependent of shear strain, which is typical for a viscoelastic rubber material. The shear modulus (G*) varies from 1.8 to 0.5 MPa. The loss modulus (G″) increases slightly with the shear strain, therefore the dissipative energy during loading, mainly bolus transition, reaches at ~1-2mJ. Even though the storage modulus (G′) involved in elastic energy recovery decreases with the strain deformation, PDMS still has a good capacity to regain the initial state after deformation (Figure 7, right). The loss tangent continuously increases at values less than unit without any phase changes. That is consistent with behavior of quasi-elastic materials with high flexibility.
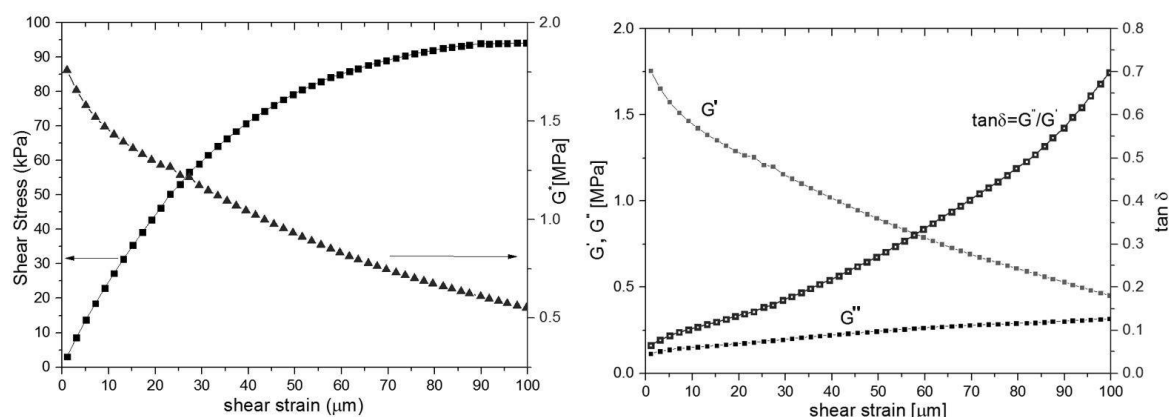
Figure 7. Mechanical properties measured by DMA, sample S1, maximum shear strain 100 microns: (left) shear stress vs shear strain and G*- shear modulus; (right) storage (G′), dissipative modulus (G″) and loss tangent tan δ.

3.3. Fatigue strength.

Samples S1, S1a and S2 are cyclically loaded and unloaded up to 150 microns, their maximum shear strain. After each 100, respective 400 cycles each sample is mechanical tested at their maximum shear strain of 100 microns. After 100 cycles samples S1 keep a rubber-elastic shear strength behavior with a rubbery plateau decreased from 90 KPa to 60 KPa (Figure 8). After 400 cycles, the sample S1 keeps elastic properties in the range of shear-strain up to 70 microns. At higher shear deformation, S1 has quite a different behavior with increased toughness. It can be hypothesized that PDMS network, during cyclic loading - unloading, reinforces by increasing self-cross-linkage between dangling bonds. Similar behavior is observed for sample S2. The elastic state reduces up to 40 microns shear strain and after this value the reinforcing increases more prominent than S1.
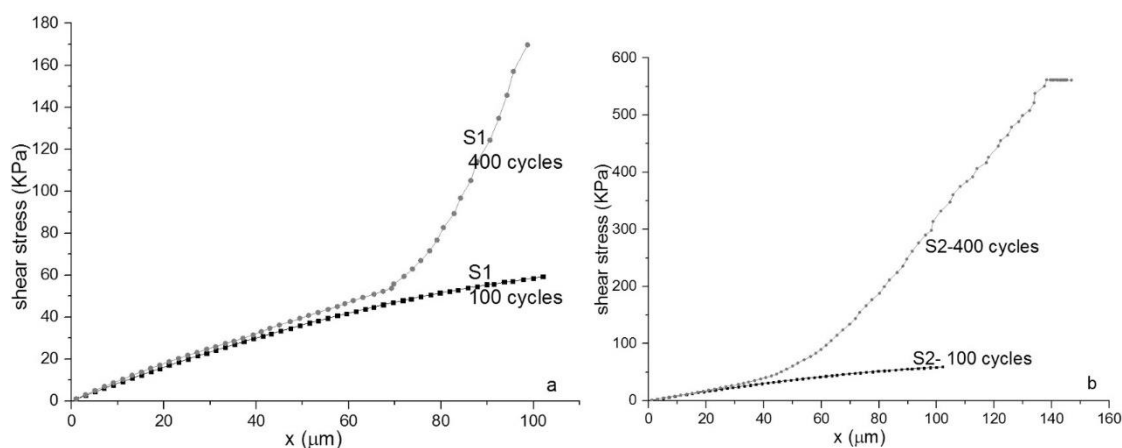

Figure 8. Mechanical tests: Fatigue strength in shear mode. Samples are loaded up to 100 µm and unloaded at shear strain rate 0.1 µm/sec. Maximum shear strain 100 µm. Toughness increases with the number of cycles tested to fatigue strength. S2-Ag nanoparticles decrease toughness.

4. Discussion

The study describes the first prosthesis designed using a combination of silicone elastomer and silver nanoparticles used for the reconstruction of the upper digestive tract. This project was based on the previously used Montgomery prosthesis which is a single silicone polymer device. Our main goal was to create an adequate polymer which can support the functions of the previous Montgomery

prosthesis improved in terms of fungus colonization. Clinical outcome is dependent on the structural design of the prosthesis and on the capability of the biomaterial that is made of.

Silicone based polymers have been used for reconstruction in the head and neck region but failed to address all the issues. Silver nanoparticles have proven their role in diminishing germ and fungus colonization of biomaterials. The blend that we used to develop the improved Montgomery prosthesis is similar to other blends that have already been used in biocompatible devices, therefore, the method is already having its applications.

Raman spectrometry and mechanical testing provided us with basic and advanced data regarding the functional parameters of the prosthesis and these are consistent to other studies involving silicone/silver nanoparticles biomaterials [36]. By using this blending technique and receipts the polymer develops a network with cross-linkage on -Si-O- with low levels of cross-linking on $-CH_2-CH_2-$ vinyl group which conferrs high levels of elasticity and, thus the possibility of high elastic energy accumulation.

Given the fact that the shear stress/shear strain curve has a quasilinear behavior up to 30 microns per millimeter reaching a plateau at shear strain ~ 100 microns we were able to say that this polymer is consistent with rubber-elastic materials. This behavior enables the polymer to return, after a deformation, to a quasi-reversible return to the initial state. All the data recovered from the shear stress/shear strain curve leads to the fact that this polymer has high flexibility which is mandatory for this type of prosthesis use.

Both samples, S1 and S2, interpreted in terms of toughness, show several features useful in biomechanical properties. In physical sense, toughness is really a measure of the energy absorbed before it breaks and estimated from the area underneath the stress-strain curve. Both samples show a transition from low toughness (high rubbery state with low energy absorbed) to high toughness where adsorbed energy is high and leads to breaking, losing the essential properties during the effort done for bolus transition.

In terms of quality of life, the reconstruction with synthetic prosthesis for most patients is well tolerated, considered satisfactory regarding silicone implant. However, some unwanted symptoms might be encountered: deglutition disorders until accommodation with prosthesis; symptoms of gastroesophageal reflux disease; foreign body sensation in the neck; rare cases of psychological intolerance.

The clinical results as shown on cohorts of patients after prosthesis implant and treated by a multidisciplinary approach, respective by monotherapy show a relatively good biocompatibility of the silicon rubber blended with silver nanoparticles.

To ensure that cytotoxicity due to silver impregnation is not posing any risks for our patients we determined the total silver levels in vitro which were below admitted levels (<1.1 µg L^{-1}). These data are consistent with results from a study made by Zhala Meran et al. who stated that prosthetic materials coated with silver nanoparticles are biocompatible with fibroblast cells [36]. According to SCHEER guidelines Montgomery prosthesis silicone elastomer mixed with silver nanoparticles meet the criteria for tolerable exposure (EN ISO 10993-17:2002) and for analytical contact conditions (EN ISO 10993-12) [37].

The statistical analysis we performed in Colțea Clinical Hospital ENT department related to the different forms of relapses of cancer concluded that only ~ 34 % of patients, over a two-year time, did not relapsed either with rigorous treatment follow-up or regular nutritional regime.

The limitation of the reconstructive method is derived from the inconsistency between mechanical properties of the Montgomery tube (given mechanical properties) and biomechanics of the esophagus for each patient. There is a wide variety of individual characteristics concerning anatomy landmarks and function preservation. The rehabilitation using this technique can not overcome the prior functional status of each patient, hence the variation in function preservation. However, clinical outcome in terms of deglutition over repeated cycles of use for the Montgomery tube coated with silver nanoparticles showed that the rehabilitation method and the characteristics of the improved prosthesis are optimal for upper digestive tract reconstruction.

5. Conclusions

Silicon rubbers used in prosthesis for a specific surgery application (esophagus reconstruction) are the most appropriate solution. The mechanical properties can be tailored by optimal recipes silicon oligomers/curing agent and curing temperatures, as shown previously. The fatigue strength, toughness, maximum shear strength are the key issues in designing Montgomery salivary silicone tube blended with silver nanoparticles with appropriate biomechanical behavior for each patient. Insertion of bacteriostatic agents, such as silver nanoparticles decreases the fatigue strength, increases flexibility and offers optimal local solution for fungi development.

Furthermore, starting from the premises that including nanoparticles of different agents is a real possibility we can state that this finding is useful in imagining a new concept to use bacteriostatic agents or other drugs for local therapy. Prosthesis design needs to indulge both clinical outcome as well as design methods and research in the field of biomaterials. Transoral insertion of a prosthesis for esophagus reconstruction after cancer surgery has improved overall survival rates and the quality of life for these patients. Further data and analysis will show eventual pitfalls of the technique.

6. Patents

Montgomery esophageal prosthesis are coded 878.3610 by Code of Federal regulations (21CFR 878). It is made of biocompatible silicon (recommended by Blue Book G95-1: ISO-10993 Biological Evaluation of Medical Devices Part1) tested for cytotoxicity, sensibility, implantation and sub-chronic and chronic toxicity. The reconstructive method is subject to Romanian patent for medical devices no. 130466 from 19.08.2014.

Conflicts of Interest: The authors declare no conflict of interest.

References

1.   Clark, G. Cochlear Implants: Fundamentals and Applications; Springer: Berlin, Germany, 2006
2.   Chugay, N.V.; Chugay, P.N.; Shiffman, M.A. Body Sculpting with Silicone Implants; Springer Science & Business Media: Berlin, Germany, 2014.
3.   W W Montgomery, S K Montgomery. Montgomery thyroplasty implant system. Ann Otol Rhinol Laryngol Suppl. 1997 Sep;170:1-16.
4.   J D Cooper, T R Todd, R Ilves, F G Pearson. Use of the silicone tracheal T-tube for the management of complex tracheal injuries. J Thorac Cardiovasc Surg. 1981 Oct;82(4):559-68.
5.   Liu Q, Shao L, Fan H, et al. 2015. Characterization of maxillofacial silicone elastomer reinforced with different hollow microsphere, J Mater Sci. 50: 3976- 3983.
6.   X. Sun, H. Sun, H. Li, H. Peng. 2013. Developing Polymer Composite Materials: Carbon Nanotubes or Graphene, Wiley online library. 25(37): 5153-5176.
7.   W.F. Smith and J. Hashimi. 2011. Foundation of Materials Science and Engineering, Fifth Edition, McGraw -Hill.
8.   L. Yua, K. Deana, L. Lib, L. Yu. 2006. Polymer blends and composites from renewable resources, Prog. Polym. Sci. 31, pp. 576-602.

9. Abdulakh K. Mikitaev, Mukhamed Kh. Ligidov and Gennady E. Zaikov. 2006. Polymer, Polymer Blends, Polymer Composites and Filled Poymers: synthesis, properties and applications, Nova Science Publishers, Inc. New York.

10. Nouri Al-qenae. 2010. Nano ceramic fiber Reinforced Silicone Maxillofacial Prosthesis, M.Sc. thesis, Indiana University, Dentistry.

11. Sara M. Zayed, Ahmad M. Alshimy, and Amal E. Fahmy. 2014. Effect of Surface Treated Silicon Dioxide Nanoparticles on Some Mechanical Properties of Maxillofacial Silicone Elastomer, International Journal of Biomaterials, Article ID 750398, p. 7.

12. Dhuha A. Shakirand Faiza M. Abdul-Ameer. 2018. Effect of nano-titanium oxide addition on some mechanical properties of silicone elastomers for maxillofacial prostheses. Journal of Taibah University Medical Sciences. 13(3): 281e290.

13. Eric Luis, Houwen Matthew Pan, Swee Leong Sing, Ram Bajpai, Juha Song, Wai Yee Yeong. 3D Direct Printing of Silicone Meniscus Implant Using a Novel Heat-Cured Extrusion-Based Printer. Polymers 2020, *12*(5), 1031.

14. Lee, W.S.; Yeo, K.S.; Andriyana, A.; Shee, Y.G.; Mahamd Adikan, F.R. Effect of cyclic compression and curing agent concentration on the stabilization of mechanical properties of PDMS elastomer. Mater. Des. 2016, 96, 470–475.

15. Williams, D.F. Biomaterials, 2009, 30(30), 5897,

16. Williams, D.F. Essential Biomaterials Science, ed CUP, 2014.

17. Williams, D.F. Biomaterials, 2008, 29(20), 2941,

18. Cohen, S., Green, F.E. The Journal of Clinical Investigation, 1973, 52, 2029.

19. Sevilla García, M.A., Suárez Fente, V., Rodrigo Tapia, J.P., Llorente Pendás, J.L. Acta Otorrinolaringol Esp. 2006, 57(10), 467,

20. Lörken, J., Krampert, R.J., Kau W.A, Dysphagia, 1997, 12(2), 79

21. Holinger, P.H. Laryngoscope, 1975, 85, 322,

22. Popescu, C.R. Tratamentul complex al cancerului de laringe. Editura SAS, Bucuresti, 1994.

23. Harrison L.B., Sessions R.B., Waun Ki Hong. Head and Neck Cancer: A Multidisciplinary Approach (3rd ed.) Lipppincott Williams & Wilkins., Philadelphia, 2009.

24. Bertesteanu, S.V.G., Popescu, C.R., Grigore, R., Popescu, B. Chirurgia, 2012, 107(1), 33.

25. Carlson, G.W., Schusterman, M.A., Guillamondegui, O.M. Ann Plast Surg. 1992, 29(5), 408,

26. Agasti, N., Kaushik, N.K., American Journal of Nanomaterials, 2014, 2(1), 4,

27. Cirstoiu, C., Ene, R., Panti, Z., Ene, P., Cirstoiu, M. Particularities of Shoulder Recovery After Arthroscopic Bankart Repair with Bioabsorbable and Metallic Suture Anchors. Materiale plastice. 2015, 52(3), 361-363.

28. Nica, M., Cretu, B., Ene, D., Antoniac, I., Gheorghita, D., Ene, R. Failure Analysis of Retrieved Osteosynthesis Implants. Materials. 2020, 13(5), 1201.

29. Lazar, V., Chifiriuc, M.C.: Architecture and physiology of microbial biofilms, Roumanian Archives of Microbiology and Immunology, 2010, 69(3): 92–98.

30. Costerton, J.W., Lappin-Scott, H.M.: Behavior of bacteria in biofilms. Asm News, 1989, 55: 650–654.

31. Carpentier, B. Les biofilms (2). Bullentin Societe de Francaise Microbiologie, 1999, 14(2): 105–111.

32. Donlan, R.M., Costerton, J.W.: Biofilms: Survival mechanisms of clinically relevant microorganisms, Clinical Microbiology Reviews, 2002, 15: 167–193.

33. Grumezescu, A.M.: Essential oils and nanotechnology for combating microbial biofilms. Curr. Org. Chem., 2013, 17(2):90-96.

34. Ene R., Sinescu R.D., Ene P., Popescu D., Cirstoiu M.M., Cirstoiu F.C. Proximal tibial osteosarcoma in young patients: early diagnosis, modular reconstruction. Romanian Journal of Morphology and Embriology. 2015, 56(2), 413-417.

35. Popescu, D., Ene, R., Popescu, A, Cirstoiu, M, Sinescu, R, Cirstoiu, C. Total hip joint replacement in young male patient with osteoporosis, secondary to hypogonadotropic hypogonadism. Acta Endocrinologica, 2015, 11(1), 109-113

36. Zhala Meran, Alexandros Besinis, Tracy De Peralta, Richard D Handy. Antifungal properties and biocompatibility of silver nanoparticle coatings on silicone maxillofacial prostheses in vitro. J Biomed Mater Res B Appl Biomater. 2018 Apr; 106(3):1038-1051.

37. SCHEER GUIDELINES. 18 June 2019, pg. 16-17.

38. Clarson, S. J., Semlyen, J.A. Siloxane Polymers. (Eds.) Prentice Hall, Englewood Cliffs, NJ, 1993.