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Original paper

Development and preliminary characterisation of novel textiles for abdominal hernia repair

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Abstract

Abdominal hernia repair is routinely done using surgical meshes. So far, no ideal hernia mesh exists and the majority of investigations are focused on the analysis and implementation of a wide range of materials: meshes with different fiber size and porosity, a variety of manufacturing methods, and certainly a variety of surgical and implantation procedures. Currently, surface modification methods and also development of nanofiber-based systems are actively being explored as areas of opportunity to retain material strength and increase biocompatibility of available meshes. Within this line of thought, we have developed novel polyester (PES) and polyamide (PA) surgical meshes for abdominal hernia repair and we have shown they have good biocompatibility.

Keywords

Nanofiber, hernia mesh, electrospinning, reinforce.

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Introduction

A hernia is a protrusion or projection (prolapse) of an organ through the wall of the cavity where it is normally contained. Hernias are classified based on their physical location, with the abdominal wall being the most susceptible site (KLINGE, 2012). Specifically, reports show that the most frequently encountered hernia is the inguinal hernia (70-75% of cases), followed by femoral (6-17%) and umbilical (3-8.5%) hernias. Hernias may occur in other sites such as the ventral or epigastric hernia, located between the chest cavity and the umbilicus. Generally, a hernia can be accompanied by severe pain, which worsens during bowel movements, urination, heavy lifting, or straining.

Occasionally, the protruding tissue may swell and become incarcerated leading to strangulated hernia. Strangulation will interrupt blood supply and promote necrosis, infection, and potentially life-threatening conditions. Today, many surgeons agree that use of a prosthetic mesh is the preferred way to repair hernias. Usually, the success of hernia repair was evaluated in the past based on the strength and permanency of the mesh itself, not on the degree of scar tissue or other factors, which subsequently develop in and around the mesh (STODDARD, 2016).

The surgical meshes employed for hernia repair exhibit many but not all of the desired characteristics (ZHANG, 2005; BROWN, 2007). Firstly, the ideal mesh should be able to be held in situ by peripheral sutures, resist the possibility of loading under biaxial tension (coughing or lifting actions) without failure especially during the early postoperative period. Additionally, it should promote a fast and organized response from fibrous tissue with minimal inflammation. Therefore, current research efforts focus on providing potential solutions that range from the utilization of novel materials to new designs that could ameliorate existent shortcomings (DAVID, 2008).

Nowadays, given the vast number of post-surgery complications such as mesh rejection, fibrosis, infection, adhesions, and hernia recurrence, the majority of investigations are focused on the analysis and implementation of a wide range of materials: meshes with different fiber size and porosity, a variety of manufacturing methods, and certainly a variety of surgical and implantation procedures. Nowadays, surface modification methods and development of nanofiber-based systems are actively being explored as areas of opportunity to retain material strength and increase biocompatibility of available meshes (LI, 2007; COREY, 2007).

Within this line of thought, we have developed novel polyester (PES) and polyamide (PA) surgical meshes for abdominal hernia repair and we have shown they have good biocompatibility.

Materials and Methods

Biocompatibility analysis

MTT assay is a quantitative method which allows evaluation of both cell viability and proliferation. Briefly, HCT-8 epithelial cells were incubated with 1 mg/ml [3-(4,5-dimethylthiazol-2-yl)]-2,5-diphenyltetrazolium bromide (MTT) solution for 4 h in the dark, at 37°C. After incubation, formazan crystals were solubilized with HCl-SDS, resulting in purple solution, quantified by spectrophotometry at 570 nm, using FlexStation3 (Molecular Devices, USA).

Mesh cytotoxicity exerted on the cells was investigated using LDH test (Tox7 kit, Sigma-Aldrich), according to manufacturer's instructions – cells that no longer have membrane integrity release lactate dehydrogenase (LDH) into the culture medium. In our experiment, the medium was collected and mixed with the kit's components in order to be evaluated 4 days of culture by spectrophotometric measurement at 490 nm.

For qualitative analysis of biocompatibility, cells were stained with fluorescein diacetate (FDA) and cell morphology was analysed by microscopy (Carl Zeiss AxioScope, Jena, Germany) and then processed with Zeiss Zen 2010 software. Statistical analysis was performed using Graph Pad Prism 6.0 software, Unpaired t-test. Statistically significant values were considered for $p < 0.05$.

Bacterial adhesion to meshes

Staphylococcus aureus ATCC 25923 was cultured on tryptic soy agar (Thermo Fisher Scientific, Waltham, MA, USA) for 18 h prior to the experiment. Subsequently, one colony was resuspended in 3 mL of tryptic soy broth media (MP Biomedicals, Solon, OH, USA) followed by incubation at 37 °C (18 h, 200 rpm). The suspension was adjusted to an optical density of 0.52 (at OD 562 nm) which corresponds to 10^9 colony forming units (CFU) per mL. The inoculum was further diluted to 10^6 CFU/mL in simulated body fluid supplemented with 10% fetal bovine serum. The samples for testing-t were plated onto the wells of a 24-well plate. Next, 1 mL of the 10^6 CFU/mL solution of *S. aureus* in simulated body fluid was pipetted into each well, while ensuring complete submersion of the samples. Samples were kept at 37°C at 5% carbon dioxide to allow biofilm formation. After 24 h, excess bacteria were aspirated, and the samples were thoroughly washed with PBS (three times) to ensure the removal of all media residue and non-adherent bacteria. Samples were removed from the wells with a sterile forceps and immersed in 1 mL of PBS. To strip all adherent bacteria from the sample into solution, the tubes were vortexed at 3000 rpm (20 min). The resulting suspension was serially diluted in PBS in order to quantify the CFU/mL values (Preoteasa CT, 2019).

Results and Discussion

The global hernia mesh devices market size was evaluated at USD 176.9 million in 2016 and is projected to grow with CAGR of 1.3% over the forecast period. Smoking, poor nutrition, genetic factors, and changes in lifestyle are some of the factors contributing to the elevated incidence of hernias (Industry report, 2014-2025). High incidence of hernia globally and improved patient outcomes by using meshes, which reduce operative and recovery time, are some of the factors contributing to

growth. Furthermore, some of the advantages of using meshes in hernia repair are reduced chances of recurrence and alleviated pain, which are expected to accelerate the market growth.

Warp-knitted fabric consists of a number of consecutive courses of loops, called stitches. Warp-knitted structures are manufactured from multi yarn systems whereby the number of separate strands of yarn equals the number of stitches in a row (Figure 1). In contrast to knitted structures warp-knitted structures can be trimmed and sewed.

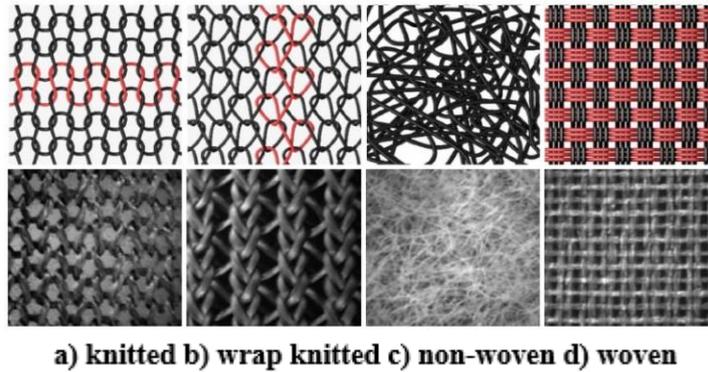


Figure 1. Textile structures

The identification of the areas with high risk of destruction was achieved by using a specialized simulation program that highlighted that the most vulnerable area is the suture (Figure 2 a-e). The stages of the modeling process included: i) Sketching mode with input data, respectively; ii) Sketcher: dimensional constraints: – greater radius: 150 mm; – minimum radius: 100 mm; constraints analysis – geometry status: closed; Part Design mode: through which the 3D profile was realized with the introduction of dimension 3 – thickness = 0.24 mm. Simulation and structural analysis – within which, by applying Finit

Element Method (FEM), the calculations were performed and the material responses to the required demand were obtained, respectively: distributed force 150 N and surface pressure 4166 N/m² (294 N/0,07 m²). It was considered that the analyzed environment is a continuous one (Fig. 2a). Constraints were applied to the entire surface by fixing the material to the entire circumference. Translational displacement vector values were obtained: [0; 0.02 mm] – Fig. 2 b. The simulation showed that the area with the highest risk of deterioration is the suture (Fig. 2e).

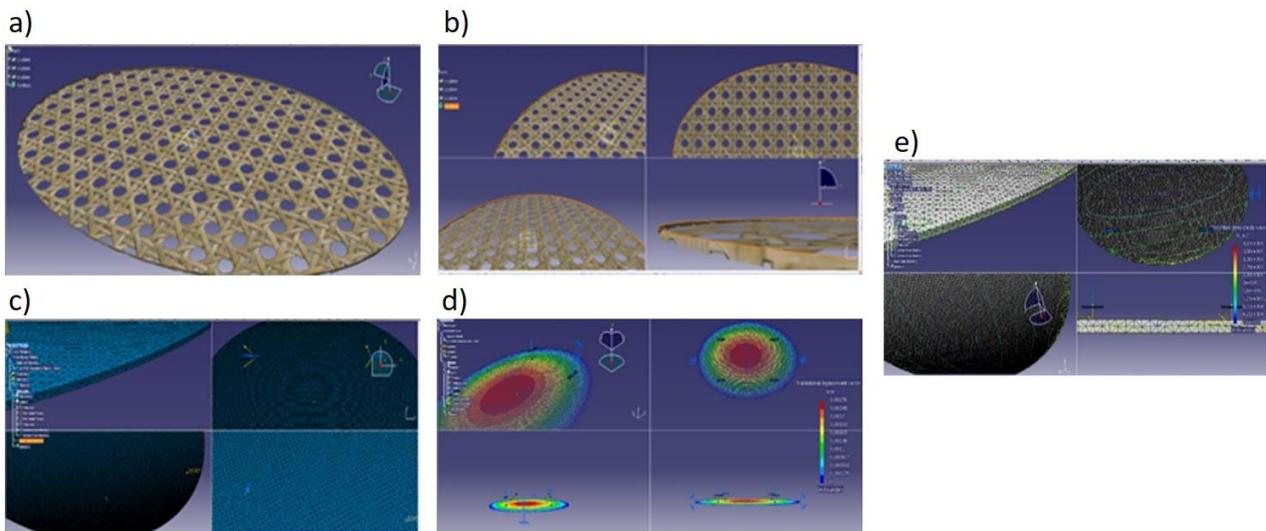


Figure 2. Mesh modeling analysis: a) Design spatial multi view; b) Part Design; c) Simulation and structural analysis; d) Translational displacement vector values; e) modal values.

Considering the intended destination, the structure knitted with threaded designs with uniformly distributed hex holes were obtained by using the combination of structures derived atlas – compound atlas derivative

(ADc-ADc), 1 full, 1 empty. The evolutions are identical and in opposite directions, all the meshes are open, with unilateral segments and the report summates vertically 6 rows of meshes.

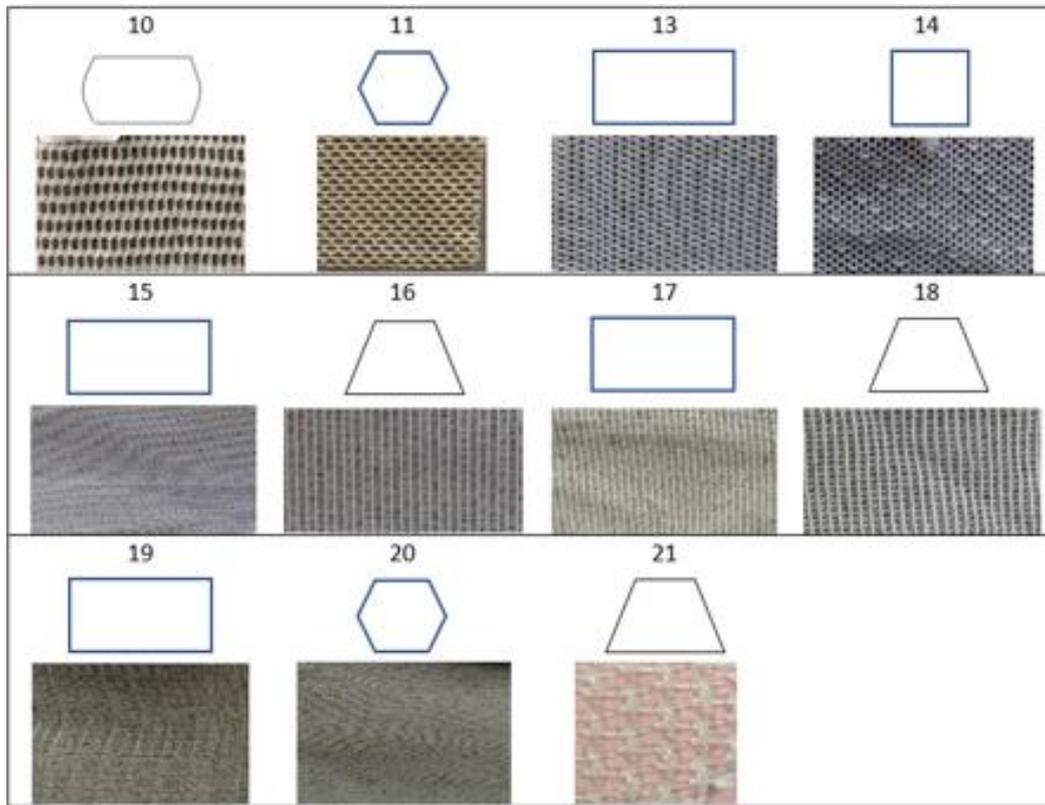


Figure 3. 11 mesh variants with various pore geometry obtained by warp-knitting technology

Figure 3 shows the obtained variants of structures with different pore geometry: square, rectangle, trapez, pentagon etc. The materials used to develop these structures are polypropylene (PP), polyester (PES) and polyamide (PA) with 76/32x2 dtex poly filamentary yarn and 0,12 mm mono filamentary yarn, while the pore size is larger than 75 μm in order to preserve tissue integration without filling the pores with scar tissue.

The main goal of our study was to develop hybrid structures between wrap-knited textiles and nano – using specific technologies while both layers of the structures will be functionalized with various substances. For instance chitosan-coated PP meshes elicited preferential attachment of myoblasts over fibroblast attachment *in vitro*, which was associated with the restoration of functional skeletal muscle with micromorphological characteristics that resemble native muscle *in vivo* and degradable drug delivery coatings with incorporated antibiotics provide a specific approach to reduce post-surgical infections.

Table 1 shows the main characteristics of the materials that were obtained. For instance, the weight of polyfilamentary yarns is situated between 49-91 g/m^2 , while that of monofilamentary yarns is between 19-107 g/m^2 . The horizontal pores density has the highest value of 80/10 cm at the square shape and the smallest value of 19,6/10 cm for pentagon shape in case of multifilamentary yarns. For mono filamentary yarns the highest value is 116/10 cm and the smallest value is of 38/10 cm. The thickness of yarns was also measured and it is situated between 0,25-0,83 mm for multifilamentary yarns and 0,18-0,64 mm for monofilamentary yarns. Resistance of bursting has values between 105,5-251,7 KPa for multifilament polyester and between 105,1-251,5 for mono filamentary yarns.

In order to establish the quantitative biocompatibility of novel hernia meshes, a MTT assay, a LDH – quantification of dead cells and a FDA staining analysis were performed.

Table 1. Characteristics of obtained structures

No.	Characteristics		UM	Variants										
				10	11	13	14	15	16	17	18	19	20	21
1	Fiber composition		%	pes	Pes	pes	pes	pes	pes	pes	pes	pa	pes	pp
2	Length density yarns	Polyf.	Dtex/den	75,0/ 67,5	150,0/ 136,3	75,0/ 67,5	150, 0/ 136,3	75,0/ 67,5	75,0/ 67,5	75,0/ 67,5	150,0/ 136,3	-	-	-
		Monof.	mm	-	-	-	-	-	-	-	-	0,124	0,040	0,156
3	Weight		g/m ²	63,0	78,0	91,0	74,0	49,0	51,0	50,0	54,0	79,0	19,0	107,0
4	Pore density	DO	Nr/pores /10cm	23,2	19,6	32,0	54,0	80,0	72,0	70,0	53,6	38,0	116,0	51,0
		DV		22,0	17,4	22,0	40,4	174,0	49,4	129,0	72,0	73,0	58,0	59,0
5	Thickness		mm	0,72	0,83	0,71	0,56	0,27	0,29	0,25	0,42	0,63	0,18	0,64
6	Resistance to bursting / deformation		Kap	133,6	105,5	170,1	141,8	242,1	251,7	206,1	168,3	251,5	105,1	283,5
			mm	24,4	25,9	32,9	31,0	35,1	29,3	27,1	30,7	33,9	28,9	18,2
7	Maxime force	Do	N	190,2	38,4	185,8	35,2	195,8	188,73	233,13	31,18	29,75	36,8	33,5
		Dv		210,5	301,2	220,5	315, 4	216,7	216,19	221,04	292,24	201,95	245,7	223,5
8	Elongation	Do	%	36,2	38,4	37,5	58,2	36,4	39,98	40,07	60,59	17,53	15,3	18,2
		Dv		14,8	21,2	17,5	21,7	17,1	15,93	13,17	23,27	27,82	18,7	16,5
9	pH aqueous extract	-	note	6,99	7,03	7,11	7,14	7,05	7,08	6,78	7,11	6,49	7,11	6,98

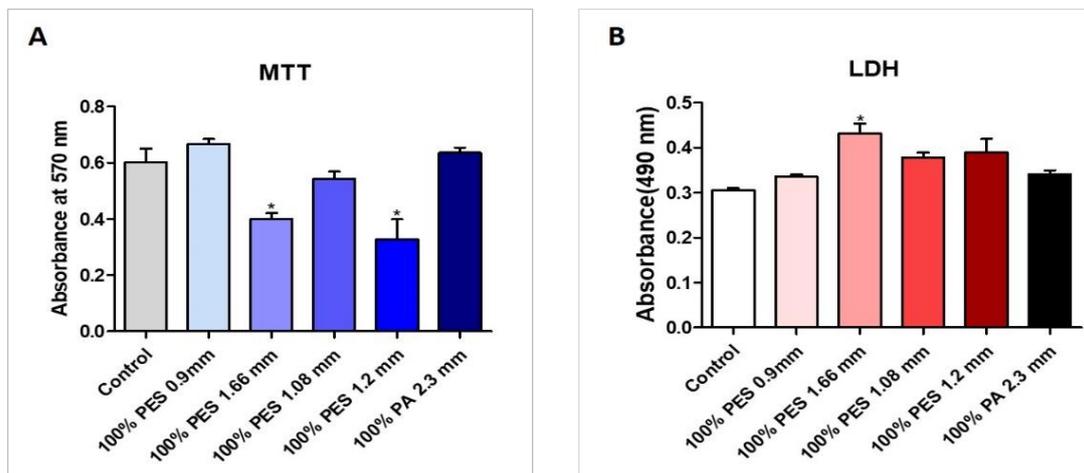


Figure 4. Quantitative biocompatibility analysis of novel hernia meshes. **a)** MTT assay – proliferation of HCT-8 cells on PES and PA meshes with various pore sizes; **b)** LDH – quantification of dead cells – HCT-8 cells grown on PES and PA meshes with various pore sizes

Fig. 4. and Fig. 5 show that the PES mesh with 0.9 mm pore size harboured the highest level of biocompatibility – with high MTT levels and LDH values comparable to the control. In addition, these results were confirmed by the microscopy analysis since cells grown on PES 0.9 mm exhibited unaltered morphology and

high density. Good biocompatibility results were obtained also for the PA 2.4 mm mesh.

Biocompatibility is a strong contributor in the rejection of the prosthesis due to scar tissue triggered by the immunological system. When a surgical mesh is implanted and lacks appropriate biocompatibility (either due to the

material that it is made of or its structural design) the body responds by encapsulating the foreign system hence leading to the formation of a stiff scar which consequently results in poor tissue incorporation, causing hernia recurrence or infection of the mesh. Consequently, a large percentage of meshes need to be removed. Importantly, approximately 69% of the explanted meshes are a result of

prosthesis infection (NIEKRASZEWICZ, 2007) mostly due to *Staphylococcus aureus* (DRAGULESCU et al, 2019).

We have investigated the *S. aureus* adherence on the newly developed meshes and have observed that meshes with small pores (PES 0,9 mm, 1,08 mm) tended to harbour higher bacterial loads compared to the meshes with larger pores (Figure 6).

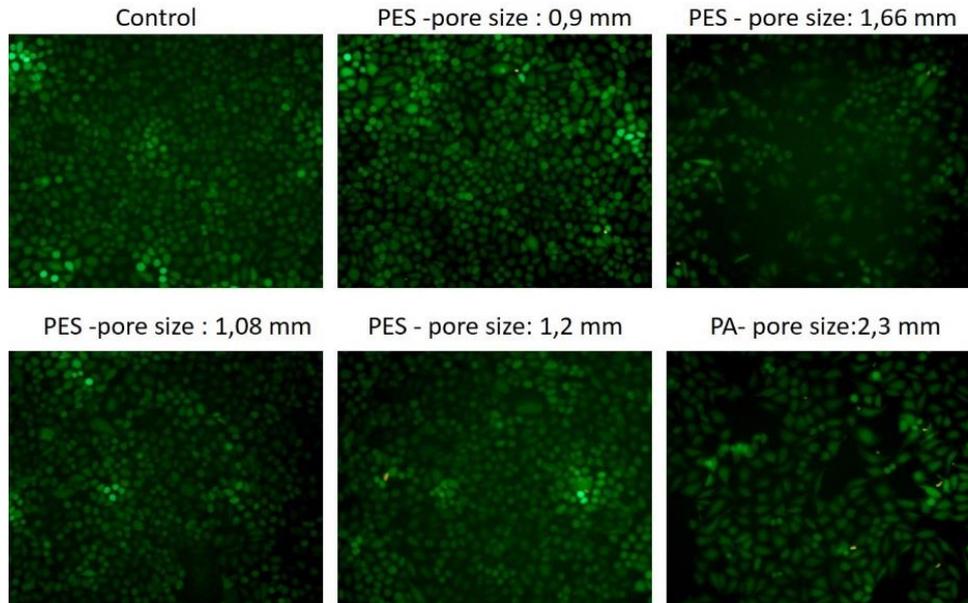


Figure 5. Qualitative biocompatibility analysis of novel hernia meshes – FDA staining (200X)

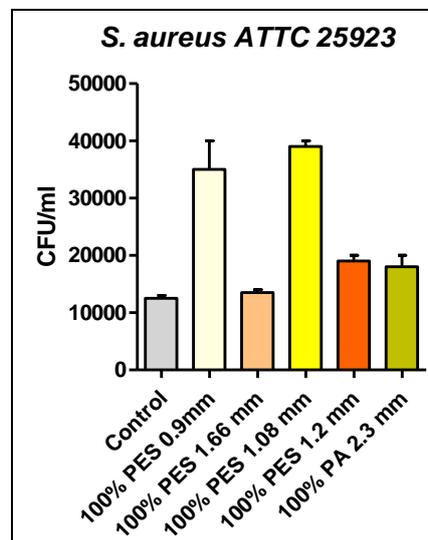


Figure 6. *S. aureus* adherence to newly developed hernia meshes

Conclusions

Nowadays, increased life expectancy leads to a proportionately older surgical population with weaker tissues. Hence, preserving the native tissues of the patient highly

challenging for the medical professionals. Consequently, there is an urgent need to develop novel biomaterials to aid the healing of the compromised native tissue. The market is saturated with many variants of surgical meshes but still the perfect hernia mesh does not exist. Moreover, the manufacturing process employed to develop these

materials are complex and increasingly expensive. Further research is needed in order to tailor biocompatible materials with optimal patient outcomes.

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