Author's response to reviews

Title: Effect on the tensile strength of human acellular dermis (Epiflex(R)) of in-vitro incubation simulating an open abdomen setting

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Author's response to reviews: see over
Dear Editor, dear Reviewers,

first we would like to thank you for your kind and rapid reviews of the manuscript. You will find our answers to the reviewers’ comments below.

Editorial requirements:

1. Please include statement in Methods that institutional ethics committee waived approval for use of blood, urine & endoscopy secretions in this study
The hAD is of human origin. But it is already approve as a drug by the German medical authorities and available on the market. We therefore are not in need of an ethic committee approval for the hAD itself. The urine was donated voluntarily and was pooled. Therefore, we did not require ethical approval. This is supported by the head of the institutional ethics committee (Prof. Dr. med. Jens P. Striebel, Head of “Ethik Kommission II der Medizinischen Fakultät Mannheim”, Maybachstrasse 14, 68169 Mannheim, Germany, Email: ethikkommission-II@medma.uni-heidelberg.de)
The upper GI secretion was from suction device reservoirs used during upper GI endoscopy. These secretions are routinely drained for inspection of the mucosa during the examination and are discarded. We do not recognize any ethical issues associated with use of the pooled waste material. This is supported by the head of the institutional ethics committee (Prof. Dr. med. Jens P. Striebel, Head of “Ethik Kommission II der Medizinischen Fakultät Mannheim”, Maybachstrasse 14, 68169 Mannheim, Germany, Email: ethikkommission-II@medma.uni-heidelberg.de)
Blood cells and fluids were voluntarily donated. All donors signed an informed consent that part of their donation may be used for research purposes. Furthermore, the cells used for our investigation are obtained from units that would otherwise be discarded since they are not transfusable. (please refer to Ethic Approval 87/04 of the Ethik Kommission II der Medizinischen Fakultät Mannheim”, Maybachstrasse 14, 68169 Mannheim, Germany, Email: ethikkommission-II@medma.uni-heidelberg.de)
An amendment regarding the blood used was made in the Material and Methods sections.
1. Another limitation of the study is that Epiflex is not compared with other human acellular dermis products, such as allodermis. For the potential user this would make the results more valuable.

*** We do not recognize this as a limitation of the study. This initial study was intended to characterize the effects of incubation in biological solutions on acellular dermis. We used Epiflex® as a model allograft. However, we agree that a comparison of acellular dermis materials with regard to their durability in biological environments would make an interesting paper.

The human acellular dermis transplant Epiflex® is the only allogenic dermis transplant available in Germany and is the only allogenic dermis transplant that is approved as a medicinal product in Europe. Alloderm® would be only legally available in European countries that do not regulate allografts as medicinal products and only then in those countries for which a marketing registration as a human tissue product. As far as we are aware Alloderm has no such registered status. We are monitoring the approval status of dermis allografts with regard to inclusion of additional products in our work.

2. In addition, the authors should pay attention in the discussion to what is the mechanism causing the lower mechanical strength, enzymes, lower pH? Also the authors may describe what is present in the upper GI secretion. I was somehow suprised that Ringers only also caused lower strenght whtin 3 weeks.

*** We agree with the reviewer. A corresponding paragraph was added into the discussion section. Please refer to answer to second reviewer #4 as well.

Added paragraph:

The decrease in mechanical strength in the different liquids might be caused by various factors. Upper GI secretion is a mixture of gastric fluid, bile and pancreatic fluid and contains a heterogeneous mixture of digestive enzymes including proteases, lipases and amylases [1, 2]. The upper GI secretion was frozen shortly after collection at -80°C to retard reduction of enzyme activity. Since the extracellular matrix consists of various proteins, glycoproteins and polysaccharides, enzymatic hydrolysis would seem to be a likely contributor to loss of tensile strength [3, 4]. Bacteria also secrete hydrolytic enzymes such as collagenases, whereby the extent and the composition depends on species and strain. Furthermore, microbial organisms can modify the pH of the environment [5-7]. This may influence the degradation of bioresorbable materials [8-10]. It is known that superoxide ions from leukocytes and macrophages accelerate the degradation of absorbable materials [11]. The mechanism leading to loss of mechanical strength in Ringer’s solution after 21 days is unclear. Temperature may affect biodegradation [12, 13], however the incubation temperature in our study (37 °C) seems unlikely to have exacerbated hydrolysis. Numerous studies demonstrated a significant loss in strength of biodegradable materials in aqueous solutions, presumably by cumulative low-level irreversible hydrolysis [10, 13, 14]. But it has to be considered that the tensile strength of hAD in Ringer’s solution at day 21 is still higher that the tensile strength of the current alternative Vicryl Mesh at day 0 (25MPa vs. 3.9MPa) [15].
3. In the histology sections, could there be also effects on elastin fibres next to the collagen? And are bacteria attached to fibres present?

*** It is safe to assume that elastin and other proteins will be affected in a broadly similar manner to collagen. However an investigation of the effects of the incubation environments on matrix components other than collagen was not part of this study. We used collagen autofluorescence to visualize the collagen structure after incubation.

*** We did not look for attachment of bacteria to matrix fibres.

**Discretionary Revisions**

1. **Abstract and Introduction.** It would be more clear if the abbreviation GI was explained in the text also (like for human acellular dermis, hAD).

*** The corresponding section in the manuscript has been revised.

2. **Introduction. page 2.**
   It is stated that Epiflex is approved for use as a medicinal product in Europe. Why then the phase I and III study that are mentioned in the discussion? Or is Epiflex only approved for limited indications? Please clarify. Probably human acellular dermis products are only in Germany regulated as medicinal products, not in other member states?

*** Epiflex® is approved as a medicinal product in Germany. Only a small number of European member states regulate human tissue transplants as pharmaceuticals. In other countries ad hoc systems adhering to the European Tissue Directive and in some cases closely aligned with the Medical Device Directive are in place. As such human tissue transplants must obtain individual approvals in each member state. The clinical trials that we are planning include exploratory and efficacy studies in novel indications.

3. **Introduction page 2**
   The method that is used to remove the cells from the human dermis can also influence the mechanical strength, please add this to the factors described.

*** Epiflex is decellularized via a proprietary manufacturing process. This process is based on the use of hyperosmolar salt solutions and dilute detergent solutions. We do not consider it likely that there is any specific mechanistic association between the decellularizing solutions and the loss of mechanical strength caused by
the incubation environments.
It may be the case, that dermis decellularized by this process has different mechanical properties than non-decellularized dermis, and/or dermis decellularized by other processes. However that is not an issue here. The mechanical strength of Epiflex® and related products has been described elsewhere. In this paper we are investigating the loss of strength after long-term incubation in biological environments.

4. Method page 4: How long were the samples submersed in Ringer’s before testing, 10 min?

*** We amended the sentence to specify the procedure more clearly.

**Amended paragraph:**
The cut samples were submersed in Ringer’s solution for about 30 minutes prior to testing to prevent drying.

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**Reviewer:** Frank Pfeffer

**Reviewer’s report:**

1. Why did the authors choose human acellular dermis? To close the abdomen, the method described depends on biological characteristics of the mesh allowing direct contact with the intestine. Other biological meshes like xenogenic meshes (f.e. Permacol®) are clinically available and widely implemented in clinical use. It would be interesting to see how these meshes perform.

What is the advantage of Epiflex®?

*** The role of xenogeneic epitopes such as the galactose-α-(1,3)-galactose terminal carbohydrate epitopes (α-Gal) are currently controversially discussed in the rejection of xenogeneic graft materials [16-20], the use of a human implant can eliminate concerns about these species-specific Gal- and non-Gal glycoantigens [21, 22].

Several other commercial human decellularized ECM matrices with similar properties and compositions have been described. These include: AlloDerm (Lifecell; skin), AlloPatch® (MTF; fascia lata), Axis™ dermis (Mentor; dermis), Bard® Dermal Allograft (Bard; dermis), Graft Jacket® (Wright Medical Tech; skin) and Suspend™ (Mentor; fascia lata) [23]. However, it should be noted, that none of these materials are approved for use in Germany, Austria, or Sweden and their approval status in other European member states where human tissue transplants are not regulated as medicinal products is unclear. Epiflex® is the only human ECM that is approved as a medical product in Germany. Such a “drug” approval requires more stringent licensing and control procedures, and it could be argued that increased patient safety is a consequence [24]. Thus, it was not our intention to compare one acellular dermis to another acellular dermis. Our intention was the proof of principle and to identify conditions not suitable for a repair with acellular dermis.
2. In a clinical setting you may have a situation with more than one fluid group (f.e. blood and bacteria). Why not use all fluids in one group?

*** We agree. There might be synergistic in-vivo effects that increase the loss of mechanical strength. However, our intention was to initially establish an in vitro test system and to identify those fluids that had the strongest effects. We are planning additional work with complex incubation environments.

3. The incubation is stopped after 21 days. Even in the Ringer solution group the tensile strength seems to decrease further on. If the mesh fails after 30 days, it is not suitable.

*** Wound healing processes such as adhesions to the transplant and integration, remodeling, vascularization, inflammation and scarring will have an effect on the mechanical strength of the transplant and the forming abdominal wall. It should not be assumed that incubation in Ringer’s Solution for 21 days is representative of the clinical situation. On the other hand maximum pressures in the abdominal wall described by other authors are far lower than the tensile strength of hAD in Ringers at day 21 [15, 25-28]. The effect of long-term incubation in Ringer’s Solution merely provides a baseline against which the effects of the other incubation solutions are assessed.

4. The authors comment limitations regarding their bacterial solution. How could they be aware that the upper GI fluid is still enzymatic active?

*** As described in the materials and methods section, the secretions were pooled into volumes of 5 liters, aliquoted into 15ml tubes and stored at -80°C. At this temperature, the enzymatic activity should be retained over a long period of time. In the pooled GI secretion we used the activity of lipase was measured with 43700 IU/l and the activity of lipase was 97200 IU/l with a pH 6.

5. The microscopic analysis is not quantified. …disaggregation seems to occur more rapidly and more distinct…”is the only result mentioned in the text. Fig.4 and 5 does not explain this finding. It would be better to show one image with little disaggregation and compare it with one image with disaggregation and explain the findings in the legend. Otherwise the microscopic analysis can be removed.

*** We agree with the reviewer and the images have been modified accordingly. For better understanding, we have compared Ringer’s solution at day 21 with the bacterial group at day 21 in order to clarify the structural damage caused by the bacterial enzymes. Therefore, Figure 4 and 5 were combined into one image (Fig. 4).
We are looking forward to your kind opinion on our revised version.

With kind regards

Eric Dominic Roessner, MD  
Vice head of the Division of Surgical Oncology and Thoracic Surgery

References


