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Bioengineering of Colo-Rectal Tissue

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1. Introduction

About 30 years ago, first reports have appeared in the literature on successful substitution of gastrointestinal wall defects by synthetic materials. An either absorbable or non-absorbable polymer patch sutured into a full-thickness wall defect of stomach, small intestine, and even colon of different animal species generally showed to be initially integrated and overgrown by neomucosa, and eventually absorbed or intra- or extraluminally extruded (Thompson et al., 1986; Harmon et al., 1979; Contieri et al., 1980; Smyrnis, 1982). Though this approach has been revived in several publications reporting good results (Oh et al., 2002; Uzun et al., 2010), treatment of damaged bowel by implantation of synthetic material has never gained general acceptance in the surgical community, and is, therefore, out of common clinical practice. This may be due to the general concern regarding the use of synthetic material in tissue which is naturally colonized with bacteria, as it is particularly the case in the colon. More recently, also natural materials like collagen sponge or acellular matrix have been tested for their ability either to support healing of an intestinal wall damage or to entirely substitute a full thickness defect. Implantation of patches of these natural biomaterials yielded good results with respect to histological and, to some extent, even functional reconstitution (Wang et al., 2005; Wang et al., 2003; Demirbilek et al., 2003; Badylak, S. et al., 2000; Isch et al., 2001; Kajitani et al., 2001; Mutter et al., 1996). As far as concerns the interposition of a synthetic or natural scaffold of tubular shape into esophagus or small intestine, results are less promising. Poor mucosal regeneration, stricture formation or high mortality rate of the experimental animals due to anastomotic leaks were reported, no matter whether the implanted biomaterial was synthetic absorbable (Thompson et al., 1986), non-absorbable (Fukushima et al., 1983; Watson et al., 1980), or of natural origin (Badylak, S. et al., 2000; Badylak, S.F., 2005; Chen & Badylak, 2001). There are no results reported in the current literature dealing with implantation of a tubular scaffold into the large intestine. In recent years there is a growing effort being done to apply tissue engineering methods for the complete, thus tubular, reconstruction of gastrointestinal organs.

A driving force of the endeavours of gastrointestinal tissue engineering is the intention to circumvent transplantation and the associated lifetime need for immunosuppression as the sole alternative to the complete absence of the respective organ. Since loss of the entire colon is absolutely compatible with life, this is not a condition that would require transplantation, indeed. However, total colectomy leads to important changes in enterohepatic circulation,

microbiology, and in water and sodium absorption (Papa et al., 1997). Furthermore, a considerable number of patients suffer from a deficient or missing anorectal continence organ due to intractable neuro-muscular dysfunction, oncological surgery or congenital malformation. The impact on patient's quality of life and the associated health care costs are considerable. Substitutes of the anorectal continence organ such as artificial bowel sphincter or musculoplasty still go along with a high morbidity and questionable functional results. Tissue engineering might have the potential to avoid some of the shortcomings related to these devices and reconstruction techniques. However, current gastrointestinal tissue engineering is clearly focused on esophagus, stomach and small intestine, whereas research on bioengineering of colon is scarce (Penkala & Kim, 2007). In this chapter we will address the different approaches that have been taken hithterto, and discuss new ground that might be broken to substitute colo-rectal tissue.

2. Different approaches for colo-rectal tissue engineering

As a matter of fact, colo-rectal tissue engineering has been struggling for its existence up to date. Given that most hollow organs are organized in a similar fashion, consisting of epithelium or endothelium on the luminal side surrounded by a collagen rich connective tissue and muscle layer, some of the knowledge gained in other fields of hollow organ tissue engineering might be successfully applied to bioengineering of large intestine.

2.1 Biomaterials

Studies on materials used with intent to bioengineer colonic tissue do not exist. However, in an attempt to prevent the feared complication of disruption of an anastomosis (a condition where two ends of the colon are sutured together in order to re-establish gastrointestinal continuity after resection of a part of the colon) many synthetic or natural biomaterials have been tested. Despite encouraging results, these biomaterials have not found their way into common clinicial practice yet.

2.1.1 Synthetic polymers

Technical aids such as the SBS-tube (Buch et al., 2002), a synthetic compound consisting of polyethylene glycol and a mixture of palmitic and stearic acid (nonattached intraluminal degradable tube) or Coloshield (Cuilleret et al., 1991), a nondegradable intraluminal Latex or silicone tube sewn to the bowel, were developped in order to support or even enhance regeneration of colonic tissue during the wound healing process. These synthetic materials act through diversion of the intraluminal fecal stream from the zone of the anastomosis rather than by exerting a direct promoting effect on the wound healing. Though, they showed to decrease anastomotic leakage and dehiscence, complications such as erosion of the tube through the bowel wall and obstruction were described(Egozi et al., 1993). Henne-Bruns and colleagues enwrapped (Henne-Bruns et al., 1990) the anastomosis with polyglycolic acid (PGA) meshes. This approach turned out to be associated with motility disorders, delayed healing and leakage followed by peritonitis. Admittedly, these materials have not been tested for colon tissue engineering purposes. However, given the considerable complication rate, they do not qualify for being further assessed in this context. Implanted expanded polytetrafluoroethylene (ePTFE) graft (Uzun et al., 2010), or Dacron (Contieri et al., 1980) used to cover full thickness wall defects in the colon showed to

function as a guide for ingrowing tissue that originated from the wound edges, thus, enabling closure of the respective tissue gap. They have nevertheless not been integrated into the regenerating tissue, but were eventually rejected. Given the inert nature of their surfaces, it remains doubtful whether they were suitable for acting as „real" tissue engineering scaffolds that would facilitate engraftment and promote growth of host tissue cells.

PGA scaffolds are successfully being used for small intestinal tissue engineering (Mooney et al., 1994; Sala et al., 2009; Sala et al., 2011). These principles were applied to bioengineering of the colon. Grikscheit and colleagues constructed 2-mm thick nonwoven PGA tubes. They were then seeded with previously harvested mesenchymal – epithelial cell compounds, derived from dissection of autologous full thickness (sigmoid) colon wall and referred to as "oragnoid units", and eventually implanted into the omentum of recipient animals. Within 4 weeks, these constructs grew to neo-colon tissue that exhibited a mucosal architechture and electrophysiological parameters comparable to that of native colon (Grikscheit et al., 2002; Grikscheit et al., 2003). A big achievement beyond doubt. However, the so bioengineered colon derives from syngeneic tissue. This would currently exclude its use for the treatment of patients with diseased autologous donor cells. Furthermore, this technique is substantially limited of what concerns customization of size and shape of the bioengieneered tissue. Indeed, tissue engineering of organoid units is the creation of a premature tissue which has to be further processed in order to become a definite, functional organ.

2.1.2 Natural scaffolds

Since interests in bowel tissue engineering are focused on small intestine this is the field where natural scaffolds have mostly been evaluated. Natural scaffolds are synonymous with extracellular matrix (ECM), consisting of interstitial matrix and the basement membrane. ECM of different origin have been evaluated for their potential to serve as scaffold for regeneration of intestinal tissue. Extensive resection of small bowel followed by implantation of lyophilized dura mater was shown to induce neomucosa on the patch, with histological characteristics similar to normal. However, the authors stated that although the creation of neomucosa on dural patches is feasible and conditions a slight improvement in the animal's nutritive status the high perioperative mortality of the experimental animals casts its applicability in intestinal tissue engineering into doubt (Hernandez Bermejo et al., 1993).

Different natural biomaterials have been studied with respect to their influence on healing of colon wall injuries. Small intestinal submucosa (SIS) was shown to be largely successful in promoting healing of a 1 cm^2 full thickness wall defect in unprepared large bowel and serving as a bioscaffold for regeneration of the native colonic tissue (Ueno et al., 2007). Another group used as what they referred to as reconstituted connective tissue patches consisting of elastin, fibronectin and collagen. Using these patches applied with fibrin sealant to the edges of a 1 cm diameter colonic defect, complete reconstitution of the mucosal as well as the circular and longitudinal muscle layer after 40 days was obtained. No retraction and inflammatory reaction were found, and the patch was slowly resorbed (Marescaux et al., 1991). Accordingly, placental-derived tissue matrix applied to a 1.5 x 2.0 cm parietal whole in the large intestine healed significantly better than fibrin glue repair alone (Alam et al., 1998).

There is a growing body of evidence that surface characteristics of the biomaterial has a determining influence on cell adhesion patterns and the ability to support and maintain differentiated phenotypes and their functions. Brown and colleagus demonstrated that three different ECMs, including SIS, urinary bladder matrix (UBM) and liver extracellular matrix (LECM) displayd distinct surface properties. These differences were reflected in their ultrastructure, as demonstrated by scanning electron microscopy (SEM) as well the distinct surface pattern of proteins and their fragments. While luminal and abluminal surface topography differs in ECM origninating from hollow organs like urinary bladder (UBM) or intestine (SIS), such a sidedness could not be observed in ECM of solid organs, like LECM (Brown et al., 2010). It remains, however, unclear whether the molecular composition or the ultrastructure of these scaffolds determine to a greater extent the outcome of the cell-scaffold interaction. The authors suggested that it was likely the diverse combination of both the structural and the functional components that accounts for the success of ECM based materials in the respective organ system (Barnes et al., 2011). Given the aforementioned aspects SIS may appear most suitable for intestinal tissue engineering. However, the ideal natural scaffold for colo-rectal constructs has not yet been defined.

2.2 Cells

Generally, the combination of biomaterial with cells was shown to improve graft integration and mucosal regeneration compared to when biomaterial is used alone. Cell seeding has been found to induce smooth muscle regeneration with less inflammatory reaction and fibroblast ingrowth as well as reduced contraction and shrinkage than is seen in unseeded grafts (Oberpenning et al., 1999; Yoo et al., 1998). Cell-cell- and cell-matrix interaction play an important role in growth and differentiation of various epithelial cell types, including urothelium (Zhang et al., 2004), oral mucosa (Izumi et al., 2003) and intestine (Kalabis et al., 2003; Rubin, 2007). Furthermore, epithelial and mesenchymal cells (i.e. smooth muscle cells [SMCs]) co-cultured on a biomaterial were shown to mutually influence their proliferation (Zhang et al., 2000; Baskin et al., 2001; Master et al., 2003). The principle of mesenchymal-epithelial interaction has also been substantiated in gut tissue (Pinchuk et al., 2010; Kosinski et al., 2010; van der Flier & Clevers, 2009; Barker et al., 2008). Apart from that, in vitro seeded cells, whether of mesenchymal or epithelial origin, might also function as a place-holder to facilitate ingrowth of host's natural epithelium on the biomaterial in vivo. This might prevent luminal bacteria from getting into contact with the underlying biomaterial, thus avoiding scar tissue formation and contraction of the graft due to infection and inflammation.

Taking into account the layered composition of hollow organs such as large intestine, smooth muscle cells and epithelial cells appear to be of pivotal interest for colo-rectal tissue engineering. Since particularly culture of intestinal epithelial cells still signifies a considerable challenge, alternative epithelial cell sources may keep on being necessary for successful colon bioengineering. It is of utmost importance that the cells used for tissue engineering do not grow in an uncontrolled fashion. Therefore, neither cancer nor immortalized cells qualify for being adopted in this context. On the other hand, primary cell cultures and stem cells seem currently to be the most reasonable of available cell sources for tissue engineering purposes.

2.2.1 Primary cell lines

2.2.1.1 Smooth muscle cells (SMCs)

Isolation and culture of SMCs were established in different species and hollow organ systems (Nakase et al., 2006; Raghavan et al., 2011; Raghavan et al., 2010a; Zhang et al., 2004; Kao et al., 1988). Raghavan and colleagues were able to successfully bioengineer a ring-shaped SMC construct, that, once subcutaneously implanted remained viable until harvest at 28 days. The muscle-ring further maintained its alignment, phenotype as well as some physiological properties of SMCs (Raghavan et al., 2010b).

Very recently, the same research group has co-cocultured SMCs originating from human internal anal sphincter with immortomouse fetal enteric neurons, which they eventually implanted in mice (Raghavan et al., 2011). This construct was neovascularized after implantation without signs of inflammation. The bioengineered muscle showed physiological function similar to normal. The cells exhibited a spontaneous myogenic basal tone, and adequately respondeded to direct electrical and chemical stimuli, such as relaxation after application of vasoactive intestinal peptide, or contraction response to cholinergic stimulation with acetylcholine. Such functional bioengineered muscle might be incorporated into the concept of a possible tissue engineered ano-rectal continence organ.

Furthermore, a physiological model of longitudinal smooth muscle tissue was developed (Raghavan et al., 2010a). In this model, the SMCs isolated from rabbit sigmoid colon and arranged in the longitudinal axis formed a highly aligned cell sheet after being seeded at high densities onto laminin-coated Sylgard surfaces with defined wavy microtopographies. The so established longitudinal muscle construct not only maintained smooth muscle phenotype and characteristics of calcium-dependence, but also electrophysiological properties similiar to its archetype.

2.2.1.2 Colon epithelial cells

Though long-term cultures of primary colon epithelial cells (CEC) have been described, they remain a challenge (Chopra et al., 2010; Bartsch et al., 2004; Booth et al., 1995). Traditionally, the method referred to as organ culture was employed to study physiology and intestinal lineage outside of an organism. Small intestinal explants of fetal tissue (few mm^2 in size) can be maintained with normal tissue organization for up to 3 weeks, allowing physiological studies on growth, differentiation, and ion transport across the epithelium (Quaroni, 1985; Pyke & Gogerly, 1985). On the other hand, adult intestinal mucosa in organ culture displayed an intense regenerative activity following an initial phase of epithelial cell loss (Ferland & Hugon, 1979; Moorghen et al., 1996). Using adjuncts like embedding on collagen gel or addition of promoting factors to the medium enabled maintaining viable tissue in organ culture for up to 28 days (Ootani et al., 2009). Yet, since organ culture techniques do not allow propagation of a specific cell (i.e. epithelial) lineage, it has limited appeal in tissue engineering applications. Attempts to generate epithelial cell cultures originating from passaged outgrowths of small tissue explants did not work well for intestinal mucosa (Chopra et al., 2010).

Tissue dissociation approaches using chelating and/or proteolytic enzymes appear to qualify better for bioengineering purposes, since specific cell lines can be obtained, held in culture, and successfully passaged. However, certain prerequisites have to be followed if culture of intestinal epithelial cells should be fruitful. After preparation with the generally used chelating agent ethylenediaminetetraacetic acid (EDTA), colonic epithelial cells

normally do not attach to the unprepared plastic dish and rapidly degenerate (Deveney et al., 1996). This is presumably due to the disruption of extracellular matrix molecules that normally would facilitate cell-cell and cell-matrix interaction. These epithelial cells much more require plating on a suitable substrate like collagen matrix or a fibroblast feeder layer, whether chemically pretreated or not, to prevent apoptosis and allow cells to replicate (Kalabis et al., 2003; Wildrick et al., 1997). If isolated crypts are prepared from colonic tissue, they also contain stem cells positive for Lgr5, an approved intestinal stem cell marker (Barker et al., 2007). This enables these cell cultures to actively replicate and to form new crypts whose cells differentiate along the crypt-villous axis. Ootani et al. could also demonstrate that the crypt-villous structure is self-organizing, and that it can be generated from a single Lgr5-positive stem cell while any non-epithelial cell type is absent (Ootani et al., 2009). Proteolytic enzymes, including trypsin, dispase, DNAse and collagenase are being applied in order to circumvent the limitations of EDTA. Among a diversity of protocols of enzymatic isolation of epithelial cells, trypsin and collagen are predominantly used (Chopra et al., 2010).

Apart from employing proteolysis, a variety of compositions of culture medium, application of growth factors and additional substances, like hydrocortisone or cholera toxin, culture conditioning with non-epithelial cells as well as the use of antimicrobial agents have been proposed in order to obtain several primary epithelial colon cell cultures of different species (Birkner et al., 2004; Deveney et al., 1996; Chopra & Yeh, 1981; Bartsch et al., 2004; Booth et al., 1995; Yeh & Chopra, 1980). Yet, since suggested concentrations of the respective substrates vary considerably between different authors, Chopra et al. stated in their recent comprehensive review that the precise combination of each substrate and additive must be defined for each system (Chopra et al., 2010).

While even long-term culture of colon epithelial cells were successfully established, their use for tissue engineering purposes have not been published up to date. Given that in vitro seeding of different types of epithelial cells on a scaffold was reported to improve integration of the implanted biomaterial in vivo (Nakase et al., 2008; Oberpenning et al., 1999; Yoo et al., 1998), we speculate that this might also apply to colonic epithelial cells. Furthermore, cell-populated biomaterials may be less prone to inflammation with subsequent fibrosis and scaffold shrinkage due to the fact that the commensal flora is prevented to get into contact with the biomaterial itself. These presumptions, however, have yet to be clarified.

2.2.1.3 Alternative epithelial cells – oral keratinocytes

Assuming that culture of colonic epithelium remains a challenge, alternative cells sources for bioengineering of large intestine are of substantial interest. Oral keratinocytes have already been successfully used in tissue engineering of epithelial cell-lined constructs. Nakase et al. (Nakase et al., 2008) evaluated healing of interposed tissue engineered esophagus in dogs. They prepared tubular composite scaffolds consisting of a PGA felt containing SMCs and, towards its luminal side, human amniotic membrane which was either populated with oral keratinocytes and a feeder layer of autologous fibroblasts or not. These scaffolds were rolled around a polypropylene tube that was 3 cm in length and 2 cm in diameter and wrapped with the omentum of the dog. Three weeks after, the now soft tubular tissue was harvested from the abdomen, and elevated into the right thoracic space as a pedicle graft to replace a 3 cm resection of the hosts's esophagus. Absence of oral keratinocytes on the amniotic membrane side was associated with stricture formation

followed by almost complete obstruction after 2 to 3 weeks. Where keratinocyte containing constructs were implanted, however, the in situ tissue-engineered esophagus showed good distensibility and the dogs remained without feeding problems through 420 days. Esophageal peristalsis transferred food to the stomach, despite the absence of peristaltic activity in the tissue-engineered esophagus graft itself. The implanted tissue-engineered esophagus displayed a histological architecture similar to that of the adjacent native esophagus. The capacity of buccal mucosa to prevent stricture formation and, thus, provide patency of bioengineered tubular constructs has been confirmed for the urethra when it was applied as either a patch graft (Li et al., 2008; Bhargava et al., 2008) or an entire tubular implant (Raya-Rivera et al., 2011). Buccal mucosa may, therefore, be a valuable alternative cell source for colon tissue engineering.

2.2.2 Organoid units

One of the few research groups who dealt with bioengineering of large intestine loaded mesenchymal – epithelial cell compounds, derived from dissection of autologous full thickness (sigmoid) colon wall and referred to as "oragnoid units", into biodegradable polymer tubes that were eventually implanted into the omentum of recipient animals. After 4 weeks, the constructs have grewn to cysts consisting of many representative elements of an intact colonic architecture including normal epithelium, vascularization, present ganglion cells, and muscularis propria. Furthermore, this neo-colonic tissue exhibited both absorptive and secretory functions. Those animals who have undergone anastomosis of the bioengineered cyst to the native colon suffered no electrolyte imbalances, showed fewer symptoms of dehydration, and had evidence of bile acid recycling as well as raised content of short fatty acids in the stool. These findings were reflected in less weight loss, less relative hyponatremia, decreased stool moisture, elevated transit times and more formed stool in animals with implanted neo-colon (Grikscheit et al., 2002). Further studies revealed that bioengineered large intestine out of previously tissue engineered colonic tissue completely retained the architectural and physiological characteristics of its archetype. Evaluation of physiological function using an Üssing chamber suggested adequate vectorial ion transport, barrier function, and viability (Grikscheit et al., 2003).

These results are promising, of course. However, the fact that the so bioengineered colon derives from syngeneic tissue would currently preclude its use for the treatment of patients with abnormal autologous donor cells, i.e. autoimmune diseases like inflammatory bowel syndrome, or types of hereditary cancer including familial polyposis or HNPCC. Furthermore, this technique is substantially limited of what concerns customization of size and shape of the bioengieneered tissue. Finally, apart from the muscularis propria, smooth muscle layers that might render peristalsis possible are completely absent. Indeed, this approach facilitates the creation of a premature tissue which has to be further processed in order to become a definite, functional organ. Despite these limitations, organoids may be successfully used in combination with other techniques/biomaterials for future tissue engineering of large intestine.

2.2.3 Progenitor and stem cells

2.2.3.1 Muscle progenitor and stem cells

The transplantation of muscle progenitor cells (MPCs) has been evaluated as for a treatment of genetic and acquired muscle disorders (Gussoni et al., 1999; Leobon et al., 2003; Yiou et

al., 2003b). MPCs are considered quiescent adult stem cells and are located under the membrane surrounding the muscle fibers. After trauma or damage, MPCs drive muscle tissue regeneration by proliferating and differentiating into myoblasts, further fusing and eventually forming new myofibers. While entire muscle naturally contains cells of different origins, including from the vascular and hematopietic compartment, MPCs represent a subset of muscle cells that are generally committed to the myogenic lineage (Benchaouir et al., 2004). Therefore, MPCs are of particular interest for muscle engineering, and may be a valuable alternative to more original smooth muscle cells in order to restore function of defective or absent anal sphincter. The potential use of injectable cultured MPCs for the treatment of stress urinary incontinence has been investigated in experimental models (Chancellor et al., 2000; Yiou et al., 2003a; Yokoyama et al., 2001). Our own results in dogs showed that autologous muscle progenitor cells are able to reconstitute irreversibly damaged urinary sphincter function. The injected cells were able to survive and formed mature tissue within the damaged sphincter region. This approach has recently been adopted to anal fecal incontinence. Three weeks after external anal sphincterotomy, MPCs from quadriceps myofiber explants were auto-grafted by injection into the anal sphincter of rabbits. The so transplanted MPCs displayed the potential for recapitulation of a myogenic program, yielding improved objective anal measures of resting and stimulated pressures as well as of electromyographic profile (Kajbafzadeh et al., 2010).

An Italian research group reported new muscle fiber formation with increased contractility of sphincter muscle strips in rats after having injected bone marrow-derived mesenchymal stem cells into the previously injured and surgically repaired anal sphincter (Lorenzi et al., 2008). Another study suggested increased contractility of the sphincter muscle in rats after cryoinjury followed by injection of autologous muscle-derived stem cells (Kang et al., 2008). While these results did not reach statistical significance, Frudinger and colleagues (Frudinger et al., 2009) were able to demonstrate a significant clinical improvement of fecal incontinence in patients who recieved an injection of muscle-derived stem cells into their anal sphincters. None of these patients showed signs of rejection or other side effects, proving that the injection of autologous cells is feasible and safe. However, the authors described a discrepancy between clinical improvement and unchanged sphincter pressure and, therefore, recommended that further clinical studies should be preceded by bench experiments.

2.2.3.2 Intestinal stem cells

Recent advances in tracking, proper isolation and further processing of intestinal stem cells have promoted the interest in their use as a potential cell source for biotechnological applications (Umar, 2010). The idea to potentially reproduce intestinal tissue out of a single intestinal stem cell (ISC) is very appealing. As part of the aforementioned progress, specific cell culture conditions have been developed that allow the generation of long-lived organoids from either crypts or even from single ISCs of the small intestine. Sato and colleagues reported that the resulting intestinal „mini-guts“ display all the characteristics of native gut epithelium (Sato et al., 2009). Although not yet elucidated in detail, ISC differentiation and self-renewal seems to be regulated *in vivo* by adjacent mesenchaymal cells, including endothelial cells, lymphocytes, muscle cells, and particularly subepithelial myofibroblasts (Powell et al., 2005). This microenvironment which controls the stem cell fate is referred to as stem cell niche (Scadden, 2006).

In an *in vitro* culture, however, where the mesenchymal niche elements are lacking, Paneth cells – specialized intestinal daughter stem cells – have been identified to act as a multifunctional guardian of the mother stem cell supplying both bactericidal lysosymes and essential niche signals (Sato et al., 2011). It could be demonstrated that intestinal organoids, consisting of a central lumen lined by villus-like epithelium and several surrounding crypt-like domains, could be generated from one single Lgr5-positive stem cell (Sato et al., 2009; Barker et al., 2010). Alternatively, Ootani and colleagues used neonatal tissue to initiate three-dimensional intestinal organ cultures. In these long-term cultures architecture of the mesenchymal niche as well as the multilineage epithelial lining which is typical for intestinal mucosa was preserved (Ootani et al., 2009). In contrast to the sphere formation of other cultured epithelial cell types, intestinal stem cell-borne tissue arranges in an asymmetry in which proliferative and differentiated cell types are positioned in accordance to their natural locations in the epithelial lining. It has been speculated that this might be due to the presence of an appropriate niche microenvironment – provided by either available mesenchymal structures and/or Paneth cells – that facilitate local morphogen gradients, thereby generating progenitor zones that are seperate from areas with differentiated cells (Snippert & Clevers, 2011).

Current research is giving rise to a more detailed understanding of the stem cell's surrounding microenvironment and of the different signals that regulate self-renewal and maintain the balance between self-renewal and differentiation of ISCs (Umar, 2010). Accordingly, epidermal growth factor (EGF), Wnt3 and Notch have been identified as essential signals for stem cell support (Sato et al., 2011). Previous work of the same research group was able to demonstrate that the addition of EGF, the Wnt agonist R-spondin 1 and the BMP inhibitor noggin induced single Lgr5-positive stem cells plated in a Matrigel-based culture system to grow into crypt-like structures with de novo generated stem cells and Paneth cells at their bottom (Sato et al., 2009).

This fastly growing knowlegde on regulation of intestinal stem cells' growth and differentiation may stimulate new approaches for tissue engineering of both the small and the large intestine. It is a tempting idea to grow complete intestinal tissue out of a single stem cell whose growth and differentiation would be induced and regulated by the respective factors/signals tethered to the biomaterial itself. Therefore, initialization of an optimized microenvironment that immitates the respective stem cell niche as close to its archetype as possible will be one of the challenges of intestinal tissue engineering of the coming years.

2.2.3.3 Adipose-derived stem cells

Another type of adult stem cells being used in tissue engineering are adipose-derived stem cells (ADSC). ADSC have successfully been derived from fat biopsies and differentiated towards distinct mesenchymal and neurogenic cell lineages (Bunnell et al., 2008; Taha & Hedayati, 2010). Few results on generation of epithelial tissue from ADSC, including corneal (Du et al., 2010; Ho et al., 2011) and urothelium-like cells (Liu et al., 2009) as well as tracheal epithelium (Kobayashi et al., 2010) have been reported up to date. Theoretically, induction of ADSC to differentiate towards intestinal epithelial cells ought to be possible by adding the respective cocktails of chemical inducers or cytokines. This approach might be worth being pursued since the use of ADSC – like adult stem cells in general - does not imply the many socioethic issues that accompany the work with embryonic stem cells. Furthermore, ADSC are abundantly available and easily accessible by liposuction that is itself associated

with a low morbidity. Using ADSC for tissue engineering purposes also means that potentially both mesenchymal and epithelial cells can be generated out of one single cell source. However, there is still a way to go to achieve bioengineered tissue on the basis of fat-derived stem cells.

2.3 Bioreactors

Once tissue engineering has grown out of its fledgling stages initialization of an adquate vascularization of the growing tissue has arguably become its biggest challenge. Currently, construct development is often restricted to smaller than clinically relevant size due to issues of (in-)sufficient oxygen and nutrient supply. Consequently, feasibility of in vivo integration of the bioengineered tissue may be constrained (Lovett et al., 2009). Interposition of bioengineered substitutes organized in a tubular fashion, such as the gut, appear to be particularly prone to complications (Badylak, S. et al., 2000; Chen & Badylak, 2001; Thompson et al., 1986). Given that inadequate oxygen supply and decreased local microperfusion are believed to be at the origin of many adverse events in gastrointestinal surgery (Anegg et al., 2008; Attard et al., 2005; Sheridan et al., 1987), hypoxia and ischemia presumably account for anastomotic leakage and stricture formation after interposition of bioengineered tubular constructs.

In recent years, considerable effort has been done to develop bioreactors that allow nourishment of growing tissue masses in vitro whose size exceeds the largest possible unit accessible to oxygen and nutrients by sole diffusion. If successful, such bioreactors are believed to clear the way for the generation of clinically applicable, tissue engineered constructs tailored with respect to the required size and shape in vitro.

In various fields of hollow organ tissue engineering, such as of the cardiovascular system (Song et al., 2011; Iyer et al., 2011), as well as the respiratory (Tan et al., 2007) and genitourinary tract (Wei et al., 2011) the rapidly growing know-how on bioreactor design and manufacturing has ultimately enhanced their more and more frequent use. The increasing application of these devices has not yet been parallelled in gastrointestinal tissue engineering. The recent report on a perfusion bioreactor with intent to optimize conditions for long-term culture of primary intestinal epithelium can, however, be considered as an initial step towards the development of such devices in gut bioengineering. Kim and colleagues (Kim et al., 2007) designed and fabricated that bioreactor consisting of a multichannel peristaltic pump, culture medium reservoir, oxygenation and gas exchange unit, air trap, and cell-polymer construct housing unit. The appliance allows tuning of tissue oxygenation and CO_2 exchange as well as dynamic cell seeding onto tubular scaffolds. The authors could demonstrate that the dynamically seeded epithelial organoid survived for two days under flow conditions. Yet, they state that, despite the promising results, further optimizations are needed to be done regarding the parameters for dynamic seeding and the employed biomaterial itself in order to enhance cellular attachment and survival. In our opinion, if tubular bioengineered tissue of clinically applicable size ought to be successfully generated in vitro, gastrointestinal tissue engineering will not get by without efforts being performed towards development and use of appropriate bioreactor devices.

3. Conclusion

Colon tissue engineering is at its very beginning. There has been some important progress in the last decade. Yet, techniques and knowledge gained from other fields of successful

hollow organ tissue engineering should be evaluated, and if promising, applied. A lot of patients worldwide suffer from a deficient or absent colo-rectal continence organ and the subsequent impact on the patient's psycho-social condition and the related health care costs are considerable. Therefore, it seems more than justified to give weight to that field of hollow organ tissue engineering. Given the ongoing progress in biomaterial development and refinement, the rapidly growing know-how on (stem) cell culture and programming towards desired daughter cells as well as the future prospects on potential applications, such as bioreactors, we are convinced that tissue engineering of the colon is awaiting a promising future. The achievement of tailored artificial colon tissue has the potential to facilitate a great step forward in the treatment of patients after abdominoperineal resection (total excision of the ano-rectal continence organ) and, if successful, may be applied to other diseases like intractable fecal incontinence or congenital anorectal malformations.

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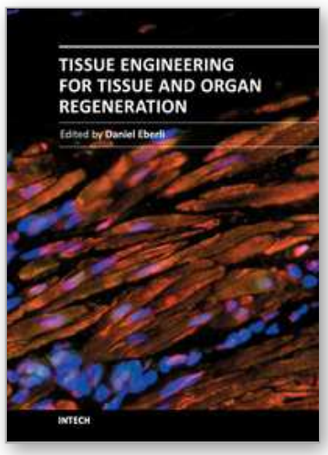

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Tissue Engineering may offer new treatment alternatives for organ replacement or repair deteriorated organs. Among the clinical applications of Tissue Engineering are the production of artificial skin for burn patients, tissue engineered trachea, cartilage for knee-replacement procedures, urinary bladder replacement, urethra substitutes and cellular therapies for the treatment of urinary incontinence. The Tissue Engineering approach has major advantages over traditional organ transplantation and circumvents the problem of organ shortage. Tissues reconstructed from readily available biopsy material induce only minimal or no immunogenicity when reimplanted in the patient. This book is aimed at anyone interested in the application of Tissue Engineering in different organ systems. It offers insights into a wide variety of strategies applying the principles of Tissue Engineering to tissue and organ regeneration.

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